AGE AND CEREAL EFFECTS ON THE PERFORMANCE, GUT PARAMETERS AND NUTRIENT UTILISATION IN THE NEWLY HATCHED BROILER CHICK

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ABSTRACT

This study was intended to provide a platform for enhanced nutritional management of modern broilers through better understanding of changes in nutritional utilisation in the newly hatched chick. Differences in performance, nutrient utilisation and the development of the gastrointestinal tract in the young broiler chicken fed diets based on different cereals were examined.

Diets based on wheat, sorghum and maize and formulated to contain iso-energy and iso-lysine levels were fed to broiler chicks between days 1 and 14 post hatch. Birds fed the maize based diet grew faster ($P < 0.05$) than those fed the sorghum based diet. Weight gains of birds fed the wheat based diet did not differ ($P > 0.05$) from those fed either maize or sorghum based diets. There were numerical differences in feed:gain with the sorghum based diet having the highest feed per gain, but the differences were not significant ($P > 0.05$). The relative weights of digestive organs and digestive tract showed no significant cereal treatment differences ($P > 0.05$). The treatments had no effect ($P > 0.05$) on the relative length of the digestive tract or gut histology measurements. The wheat based diet contained a commercial xylanase which may have ameliorated the digesta viscosity of this diet and reduced the NSP effect on gut structure and morphology. Furthermore, gut morphology samples showed a high degree of variation, suggesting that a large sample size would be necessary for significant differences to be determined.

Two experiments were conducted to investigate cereal and age effects on nutrient utilisation in the young broiler chicken. Experiment 1 determined the nitrogen-corrected apparent metabolis able energy (AME_n) of diets based on wheat, sorghum and maize during 14 days post-hatch. Changes in the total tract digestibility of starch and fat were also measured. The second experiment was conducted to confirm the results of Experiment 1 using wheat and maize based diets and was of 21 days duration. In both experiments, changes to AME_n with age were similar for all diets, declining from day 3 to days 5-9 and increasing again. In experiment 1, cereal effects were significant ($P < 0.05$) with maize and sorghum based diets having higher AME_n values than the wheat based diet. In experiment 2, cereal effects were

significant ($P < 0.05$) with the maize based diet having a higher AME_n than the wheat based diet.

In experiment 1, total tract starch digestibility determined for days 5, 7 and 14 showed no cereal differences ($P > 0.05$). Age effects were significant ($P < 0.05$) with starch digestibility declining from day 5 to day 7, and then increasing again. Total tract fat digestibility on day 7 was significantly lower for the wheat and sorghum based diets than for the maize based diet, but no cereal differences ($P > 0.05$) were observed on days 5 or 14. Age effect was highly significant with fat digestibility declining from day 5 to day 7 and increasing again. These results showed that nutrient utilisation is compromised during the first week of life of the broiler chick.

Samples of diets and excreta from Experiment 1 were analysed for minerals to determine changes in the apparent total tract mineral retention of the broiler chick during the first two weeks post-hatch. The diet and excreta samples were analysed for calcium, phosphorus, potassium, sodium, magnesium, iron, manganese, zinc and copper, and their retention was determined. The retention coefficients of individual minerals differed widely and the retentions of major minerals were much greater than those of minor minerals. The cereal effects were significant ($P < 0.05$) for several minerals, with a general tendency for the sorghum diet to have greater retention than maize or wheat diets. Age effects were significant ($P < 0.05$) for all minerals. In general mineral retention coefficients were higher at day 3, declined to day 7 and remained unchanged to day 14. Decline in mineral retention with age was similar in all three diets.

The studies reported in this thesis investigated the performance and nutrient utilisation of broiler chicks fed diets based on wheat, sorghum or maize. A significant reduction in nutrient utilisation over the period 5 – 9 days of age was observed suggesting that nutrient digestion and absorption is compromised during the first week of life of the broiler chick. Further work is required to identify the specific causes of the decline in digestibility during the early stages of chick development.

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Chapter 1

Introduction

During the past two decades the growing cycle of the broiler chicken has reduced as a result of selection for faster growth rate. Associated with this increase in growth rate has been an improvement in feed efficiency. These changes have provided the broiler industry with substantial economic advantages, particularly in relation to competing animal proteins for human consumption. There is now more emphasis on the early growth phase of the chicken, and improvements in feed efficiency over the first 14 days of age are increasingly important in achieving the full economic benefits of genetic improvements in the broiler chicken.

A detailed understanding of changes in digestive capacity and the physiological causes and effects of these changes is the key to improving bird performance over this early period. Little recent work has been undertaken in this area, and although there is a significant amount of historical information, it is unknown how accurately these data reflect the modern broiler chicken. Furthermore, a number of management changes over recent years, such as the use of exogenous feed enzymes and changes in dietary nutritional specifications, may be impacting on the post-hatch chick.

This study provides a platform for enhanced nutritional management of the modern broiler chick to improve both the market performance and economic profitability.

1.1 OBJECTIVES OF THE STUDY

Differences in growth rate and feed efficiency of broiler chickens are known to result from diets based on different grain types (Ao and Choct, 2004). Cereals contain varying levels of non-starch polysaccharides (NSP) giving rise to differing viscosity levels in both the feeds manufactured from these cereals and in the digesta of chickens fed these feeds. Increased digesta viscosity is known to reduce digestion and absorption of a range of nutrients (Annison, 1993). Wheat based diets are more viscous than maize based diets as they contain higher levels of NSP and as a result tend to be less efficiently utilised than maize based diets by growing birds. The studies reported in this thesis compared diets based on wheat, sorghum or maize, the three main grains used in the New Zealand poultry industry.

The gastrointestinal tract is a major consumer of nutrients, with rapid cell turnover utilising significant quantities of both nutrients and energy and an increase in the proportional size of the gastrointestinal tract will therefore reduce feed efficiency. High NSP cereals have been shown to increase the size of the gastrointestinal tract (Jorgensen *et al.*, 1996). The weights of the major digestive organs and of the digestive tract were measured on day 14 to assess diet related differences. NSP levels may also influence the morphology of the gut (Iji, 1999). Increasing crypt depth indicates increased villus cell proliferation, and therefore an increase in nutrients being utilised by the gastrointestinal tract. Nutrients are absorbed through the villus epithelium and changes in villus height alter the area available for nutrient absorption. This study measured both villus height and crypt depth for dietary impact.

The digestive system of the newly hatched chick is not fully developed and this impacts on the efficiency with which the chick utilises dietary nutrients. Dietary differences would therefore be expected to be more pronounced during the immediate post-hatch period. This aspect was examined in two experiments. In experiment 1, age effects on energy utilisation were measured during the first 14 days post hatch. Age effects on total tract starch and fat digestibility were also determined. In experiment 2, age effects on energy utilisation were confirmed over the first 21 days post hatch.

Published data on the changes with age in mineral absorption and utilisation in the young broiler chick are limited (Ravindran *et al.*, 2006). Accurate data on mineral retention by the young chick is necessary if diets are to meet the requirements of the chick during the early stage of development. Furthermore, increasing awareness of the environmental cost of waste disposal is placing more emphasis on minimising excessive dietary mineral levels. To assess the significance of developmental changes in mineral utilisation in the newly hatched broiler chick, mineral retention was calculated on days 3, 5, 7, 9 and 14. Both dietary and age related differences were measured.

1.2 RELEVANCE OF THE RESEARCH

Currently available published data on the nutritional requirements of the broiler chicken may not be accurate for the modern fast growing bird. Furthermore, the New Zealand broiler industry differs from industries elsewhere in a number of aspects. These include bird performance, feed efficiency and availability of feed ingredients. For these reasons, there is a need for information on dietary and age related effects in the modern broiler grown under New Zealand conditions. This study is specific to the New Zealand environment and utilised New Zealand sourced feed ingredients, and it is hoped that this study will provide the basis for more appropriate nutrition over the first 14 days post-hatch.

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Chapter 2

Literature Review

2.1 Introduction

Over the past two decades, the growing period of meat chickens has reduced markedly with the first two weeks post-hatch now comprising 40% of the grow-out period. For this reason, the early growing period of the chick is assuming increasing importance. A number of researchers have evaluated different aspects of the development of digestive capability in the newly hatched chick. Digestive organ development has been investigated by Nitsan *et al.* (1991a), Nitsan *et al,* (1991b), Nir *et al.* (1993) and Iji *et al.* (2001a). Development of gut mucosa was investigated by Dibner *et al.* (1996), Iji *et al.* (2001a), Noy *et al.* (2001), and Uni *et al.* (1998). Enzyme production has been investigated both for pancreatic enzymes (Nir *et al.*, 1993; Nitsan *et al.*, 1991a; Noy and Sklan, 1995), and brush-border mucosal enzymes (Mahagna and Nir, 1996; Uni *et al.*, 1998).

Numerous studies have been reported on the effects of early feeding on gut development and bird performance. Researchers have investigated both the impact of fasting (Halevy *et al.*, 2000; Lippens, *et al.*, 2000) and the benefits of early feeding to newly hatched chicks (Batal and Parsons, 2002b; Boersma *et al.*, 2003; Yi *et al.*, 2005). Dibner *et al.* (1996) investigated the effect of dietary ingredients on the microscopic structure of the gut. The digestion of specific nutrients including carbohydrate, fat and protein (Sulistiyanto *et al.* 1999; Noy and Sklan, 2002; Sklan, 2003) and dietary energy utilisation in broiler chicks have also been investigated (Zelenka, 1968; Sulistiyanto *et al.,* 1999; Batal and Parsons, 2002a).

Despite the number of investigations conducted on early nutrition in the newly hatched chick, there still remain a number of unanswered questions. In many cases the results reported are contradictory and suggest further research is required in order to understand the mechanisms involved, and the most appropriate management tools to maximise performance in the first few weeks of the broiler chicken's life. As the growing cycle of the broiler chicken continues to shorten, this will become even more important to the poultry industry. The literature concerning the digestive capability and nutrient digestion of the newly hatched chick are reviewed in this chapter in an attempt to determine the current scientific understanding of early chick nutrition and to identify those areas where further research is required.

2.2 Growth rate

Nitsan *et al.* (1991a) reported that the chick relative growth rate increased from 14% of bodyweight for the first seven days to 22% of bodyweight on day 11 before decreasing to 9% of bodyweight on day 17. Similar findings have been reported by Nir *et al.* (1993) who calculated that broiler chickens achieved a maximal relative growth rate of 20% at five days of age which was maintained until at least day 10 and decreased thereafter to 16 % of bodyweight by 14 days of age.

2.3 Feed intake and passage rate

Nir *et al.* (1993) found that relative feed intake peaked at 30% of bodyweight on day 3 after which it declined to 15% of bodyweight at day 15. Noy and Sklan (1995) observed that feed consumption increased three-fold between four and ten days of age, but this paralleled a 30% decline in passage rate. After 10 days of age feed intake continued to increase, however no further change in passage rate was observed. The decrease in passage time was especially high in the duodenum, the major site of activity for digestive enzymes, with a decline from 10 minutes on day four to 3 minutes on day 7, after which there was no significant change. Passage time through the small intestine declined from 161 minutes on day four to 110 minutes on day 14. Uni *et al.* (1995b) also observed a decrease in passage time over the first week of age. Passage time through the small intestine decreased from 115 minutes on day four to 74 minutes on day 7. However, in this study, passage time increased again by day 10 and reached approximately 122 minutes on day 14.

In summary, feed intake increases and passage time appears to decline over the first week of life, the period during which maximum relative growth of body mass and digestive organs occurs.

2.4 Vitelline residue

Nir *et al.* (1993) found that the vitelline residue decreased rapidly from 11% of bodyweight at hatch to 2% by day two and was negligible by day four. This finding was consistent with those of Nitsan *et al.* (1991b) who showed that 75% of the residual vitelline present at hatch is utilised by day three and that by day six vitelline residue had decreased to negligible levels and of Iji *et al.* (2001a) who measured the yolk sac at 8% of bodyweight at hatch and decreasing to less than 1% of bodyweight by day 7. Murikami *et al.* (1992) found that deutectomising chicks did not affect metabolisability of dietary energy or lipid, but delayed the growth of the chicks by two days, demonstrating the role of the residual yolk in complementing dietary nutrients. In this study, the vitelline residue was 6.4 grams at hatch, disappeared rapidly over the first three days, and was almost completely utilised by seven days of age.

2.5 Digestive organ development

After hatching the chicken needs to rapidly adapt from meeting its nutritional requirements from the yolk sac to utilising a diet based primarily on carbohydrates. To meet the nutritional needs for growth and maintenance in the developing chick, the digestive system is required to digest and absorb the exogenous diet at a rate sufficient to meet its demands. Therefore the relative growth rate of the gastrointestinal tract is greatest during the first seven days of age (Iji *et al.*, 2001a).

2.6 Relative development of intestine and digestive organs

Nitsan *et al.* (1991b) showed that the growth of duodenum, jejunum and ileum relative to bodyweight reached a maximum at 6 days of age and declined thereafter. Similar results were obtained by Nir *et al.* (1993) with maximum relative weight of the small intestine occurring at 5 days of age, and by Nitsan *et al.* (1991a) who showed a maximum relative growth rate 4-fold that of bodyweight gain at 8 days of age. Iji *et al.* (2001a) and Murakami *et al.* (1992) also showed the relative small intestine weight was maximal at seven days of age after which it declined.

Nitsan *et al.* (1991b) showed that relative liver weight peaked at 6 days of age, and relative pancreas weight peaked at 9 days of age. In another study, Nitsan *et al.* (1991a) showed an increase in relative pancreas weight to 8 days of age at which stage it had an allometric growth rate approximately 4-fold that of body growth. After 8 days of age, the rate decreased and by day 23 the allometric growth rate of the pancreas was 2.5 times that of bodyweight. In this study, the liver reached a maximum allometric growth rate on day 11 of two, and by day 15 this had declined to be similar to body growth.

Similar trends have been reported for turkey poults by Sell *et al*. (1991), although the relative growth rate of the pancreas, proventriculus and small intestine in the poults appeared to peak somewhat earlier at six days of age, after which the weight increases of the pancreas and small intestine nearly paralleled that of bodyweight. However Krogdahl and Sell (1989) found the relative weight of the pancreas in poults to peak at about 14 days of age, and to decrease thereafter, still decreasing at 56 days of age. In this study, the pancreas constituted 0.1% of bodyweight at hatch, 0.4 to 0.5% at 14 days of age and 0.2% of bodyweight at 56 days of age. Pinchasov (1995) showed that in turkey poults the relative weights of the gastro-intestinal tract, liver and pancreas increased in the first 24 hours after hatching irrespective of whether or not the birds had been fed, although the increase was greater in fed birds. A similar pattern of organ growth was observed in chicks by Murakami *et al*. (1992).

In summary it would appear that maximum relative growth rate of the digestive organs is achieved within the first 10 days of life and after this stage it declines to eventually approach that of bodyweight gain. This is consistent with the demand on supply organs caused by maximal relative body growth being achieved over 5 to 10 days of age.

2.7 Development of gut mucosa

Changes in villus height, crypt depth or submucosa thickness may influence the uptake of nutrients by the young chick. Uni *et al*. (1995b) reported that between days four and 10, villus height and perimeter increased by 25 to 100% in the duodenum, jejunum and ileum of the young chick. Crypt depth increased with age, but there were no changes in the thickness of submucosa. Uni *et al*. (1998) observed that in the first two days after hatch villus volume in the duodenum, jejunum and ileum changed little, but volume in all three segments increased rapidly thereafter. The increase was complete in the duodenum by day 7, however villus size in the jejunum and ileum increased until day 14. Increases with age were greatest in the duodenum and least in the ileum. Enterocyte numbers per villus were similar in all segments, and changed little with age. Crypt depth was greatest in the duodenum and overall increased two to threefold with age. The authors studied the effects of withholding feed for 36 hours or of deutectomy on villus volume, and reported that a decrease in jejunal villus volume was still evident after day 11 in the deutectomised chicks and those from which feed was withheld. Furthermore, crypt depth was also decreased for 8 to 10 days in the duodenum and jejunum and 5 days in the ileum, and crypt structure was abnormal between days 7 and 9.

Iji *et al.* (2001a) found that although intestinal mucosa was structurally developed at hatch, rapid growth through rapid cell proliferation, hypertrophy and an increased migration rate followed for the next several days. The rate of cell proliferation peaked at 7 days of age, and cellular migration at 14 days of age. The increase in cell proliferation may be to support both crypt and villus growth. The percentage of proliferating cells was studied by Geyra *et al.* (2001) who found all cells along the villus proliferating in all segments of the intestine at hatch. In fed birds, the percentage of proliferating cells decreased with time to plateau at 10-20% by approximately day 4. Cell proliferation was sensitive to feed intake, however re-feeding fasted birds rapidly enhanced cell proliferation. Noy *et al.* (2001) found that cell proliferation in turkey poults declined to less than 10% within 48 hours of hatch.

2.8 Mucosal DNA

A measure of cell proliferation within the mucosa can be obtained through the ratio of RNA to DNA, which gives an indication of ribosomal activity (Uni *et al.,* 1995a). Uni *et al.* (1998) found the RNA:DNA ratio to be higher in duodenal and jejunal tissues when compared to the ileal tissue, indicating greater cell proliferation. In all segments the ratio decreased with age, but at different rates. Protein to DNA ratios, which reflect cell size, were initially higher in the duodenum but decreased with age indicating increasing cell size. Overall, tissue and ribosomal activity decreased with age throughout the intestine, but at different rates. Generally duodenal tissue has the highest activity and ileal tissue the lowest, and the rate of activity decrease is lower in the ileum.

DNA content of the mucosa gives a measure of the cell population. Iji *et al.* (2001a) showed a declining DNA content in the duodenum, jejunum and ileum with age. They also found increasing cell size with age, and that protein synthesis declined with age.

In summary, rapid growth of gut mucosa through cell proliferation and cell hypertrophy occurs over the first week of life. The rate of increase varies between different segments of the gut however all reach a maximum rate of development between days 7 and 14.

2.9 Gastrointestinal tract pH

Digestive enzyme activity and microbial population is influenced by gut pH and changes may therefore impact on digestive capability. Mahagna and Nir (1996) found that gut pH measured in the crop, gizzard and small intestine dropped from day one to its lowest value on day 7, before increasing again to a peak on day 14. No subsequent changes were measured in the crop or small intestine. However, in the gizzard the pH was lower on day 21 than on day 14.

2.10 Biliary secretions and digestive enzymes in the young broiler chicken

2.10.1 Biliary secretions

Limited secretion of bile is known to be responsible for the poor fat absorption in the young chick. Noy and Sklan (1995) found that secretion of bile components to the duodenum, including bile salts and fatty acids, increased 8 to 10 fold between days 4 and 21 post-hatch.

2.10.2 Pancreatic enzymes

Chickens adjust to changes in dietary composition by altering enzyme secretion. For example, the neo-natal chick responds to dietary adjustments in starch intake by altering the amount of amylase released, and the enterocyte carbohydrase concentration (Moran, 1985).

Nitsan *et al*. (1991a) showed that the specific activity of the pancreatic enzymes trypsin, amylase and lipase decreased during the first three to six days after hatching and then increased to a level 10 to 20% higher than that of hatching on days 14, 11 and 21 respectively. Chymotrypsin activity increased from shortly after hatch to day 14. When expressed as units of activity per kilogram of bodyweight, the activity of all enzymes increased with age, reaching a maximum on day eight for amylase and lipase, and day 11 for trypsin and chymotrypsin.

Noy and Sklan (1995) measured the secretion of lipase, trypsin and amylase secretion in the duodenum of chicks aged four to 21 days of age, and also measured total nitrogen secretion to the duodenum as an indicator of total enzyme secretion. Lipase, trypsin and amylase secretions increased 20 to 100 fold from four to 21 days of age. Lipase activity increased less and somewhat more slowly than the other enzymes. Amylase secretion was relatively low at four days of age and increased rapidly with age. Total nitrogen secretion increased by 15-fold between 4 and 21 days of age.

Nir *et al*. (1993) observed that the specific activity (units/g) of amylase was highest at hatch and decreased up until day eight. Lipase activity increased from a very low level at hatch by about 40 fold on day 14. Trypsin specific activity increased gradually to reach a peak at 11 days of age, while chymotrypsin declined during the first eight days of age and followed by a marked increase. When expressed as units of activity per kilogram of bodyweight, all enzymes showed increased activity with age, reaching a maximum on day five for amylase, and around day 11 for trypsin and chymotrypsin.

In turkey poults Sell *et al*. (1991) observed that, when expressed in terms of bodyweight, the specific activities of amylase, lipase and trypsin showed moderate increases during late embryonic development. Following this, relatively large increases in the specific activity of amylase and lipase were observed on day one, with amylase specific activity continuing to increase after day two, but lipase activity not changing significantly after day one. Trypsin specific activity reached a peak on day six. Krogdahl and Sell (1989) found that the specific activities of pancreatic trypsin, protease and lipase in turkey poults increased with age after a lag period of about 14 days. They suggested that the mechanisms regulating lipase production in poults are not fully developed during the first few weeks after hatch and that lipase may be a limiting factor for lipid digestion in the early post-hatch period for turkeys. Amylase activity seemed to increase rapidly during the first 14 days, but the activity of the enzyme varied widely between samples.

In summary, although secretion of pancreatic enzymes with age varies between enzymes and studies, there is a substantial body of evidence suggesting that pancreatic specific enzyme activity (units/g) either decreases or remains stable over the first week of life. Over this period, the total enzyme production increases when expressed relative to bodyweight, but the intestine grows at a relatively faster rate than bodyweight over this period, which may confound the interpretation of enzyme activity.

Pancreatic enzyme levels in digesta

Pancreatic enzyme activity levels in the digesta may give an indication of the enzyme activity available to digest feed, and for this reason a number of researchers have measured this activity level. Nitsan *et al.* (1991a) measured digesta pancreatic enzyme activities and, found that trypsin activity increased 10-fold from hatching to day 14, and chymotrypsin activity increased 3-fold to reach a maximum at 20 days of age. Amylase activity did not change for the first two days, but then increased 5 fold by 17 days of age. Lipase activity gradually increased to reach a level 2.5 times that of hatching on day 23. Nir *et al.* (1993) observed that the specific activities (units/g) of amylase, lipase, trypsin and chymotrypsin were very low at hatch and increased with age. When enzyme activity was expressed as units per kg of bodyweight amylase peaked at 5 days of age and then declined for the remainder of the experimental period. Lipase peaked at day 7 and then declined to day 11, remaining at this level until day 14. Trypsin and chymotrypsin increased throughout the 14 day trial period. Noy and Sklan (1995) measured net amylase, trypsin and lipase secretion to the gut using ^{141}Ce. An increase in daily secretion was determined for amylase, trypsin, and lipase of 100- 50- and 20-fold, respectively, between days 4 and 21. The relative increases were lowest for lipase and highest for amylase. This study also determined changes in digestive capability over the first 21 days of age, and the authors concluded that enzymes are secreted in adequate levels for fatty acid and starch digestion in the young chick but that proteolysis may not be sufficient in the early post hatch period. However they qualify this by stating "From 10 days of age, increased feed intake was compensated for by enhanced intestinal capacity and enzyme secretion".

Krogdahl and Sell (1989) found that in turkey poults the trypsin, protease, and amylase activities followed similar patterns of development. Major increases in the activity of all enzymes were observed during the first 21 days after hatch, with no major changes in activity level observed after this time. Development of lipase activity appeared to be dependent on the fat content of the diet particularly after 21 days of age. However, even poults fed high fat diets had low intestinal content lipase activity prior to 21 days.

In summary, there is a general trend for digesta enzyme activity levels to increase over the first 21 days of age however rates of development vary between enzymes.

2.10.3 Brush border enzymes

Disaccharidases are responsible for the breakdown of disaccharides (mainly maltose) obtained from starch digestion and are attached to the brush border of the small intestine. Mahagna and Nir (1996) measured the activity of saccharase and maltase in broiler chicks and observed that the activities of both enzymes declined significantly from hatch to seven days of age whether expressed as units/gram of tissue or units/kg bodyweight. Activity levels of both enzymes remained low until 21 days of age. A study by Uni *et al.* (1998) reported that jejunal sucrase and maltase activities reached a maximum on days 1 and 2 after hatch and decreased thereafter. They also reported that mucosal sucrase and maltase activities were lower in the duodenum than in the jejunum or ileum. Iji *et al.* (2001b) found similar results, with the maximal specific activities of sucrase and maltase at hatch, and declining thereafter. However, they reported that the total enzyme activity increased with age in all intestinal segments due to an increased villus surface area and intestinal length. In contrast to the study by Uni *et al.* (1998), these authors found that total enzyme activity per villus in the duodenum was higher than the activity per villus in the jejunum and ileum as a result of the longer villi in the duodenum.

Sell *et al.* (1991) observed in turkey poults that, when expressed in units of bodyweight, the specific activity of maltase and sucrase peaked at one day of age and declined markedly through to six days of age before increasing again by eight days of age. In contrast, a previous study by Sell *et al.* (1989) reported that maltase and sucrase specific activities increased between one and seven days of age. Enzyme levels on day one were similar in both studies, however disaccharidase production then diverged. The authors could find no ready explanation for this divergence in enzyme production.

In summary, although there are some conflicting results, there would appear to be a reduction in specific disaccharidase activity over the first week of age. However increasing gut size would mean that there is an overall increase in total enzyme activity.

2.11 Comparison of growth, digestive organ development and digestive enzyme levels in fast and slow growing lines of chickens.

Several researchers have compared organ growth and digestive enzyme levels in fast and slow growing lines of chickens. Broiler chickens were compared with fast or slow growing lines of White Plymouth Rock chickens by Nitsan *et al.* (1991b). Comparisons of egg type chicks and broiler chicks were also undertaken by Nir *et al.* (1993), Uni *et al.* (1995b) and Mahagna and Nir (1996).

2.11.1 Bodyweight

Nitsan *et al.* (1991b) found that at hatching the difference in bodyweight between broiler chickens and slow growing Plymouth Rock chickens was less than twofold, with the fast growing Plymouth Rock chickens intermediate. This general pattern continued to 15 days of age by which time the difference in bodyweight between the two extremes increased to eightfold. Nir *et al.* (1993) found that broiler chickens reached a maximum relative growth rate of 20% at day five which was maintained for a further five days, before declining to 16% on day 14. In contrast, the egg type chickens reached a peak of 14% relative growth rate at 4 days of age, before declining to 5% at 14 days of age. Uni *et al.* (1995b) and Mahagna and Nir (1996) found similar patterns of growth when comparing light and heavy strains of chickens.

2.11.2 Feed intake

Nir *et al.* (1993) found that food intake per unit of bodyweight attained a peak of 30% at day three for the broiler chicks and 20% at day six for egg type chicks. In both types, relative food intake then decreased to 15% of bodyweight by day 14. A similar pattern of feed intake was reported by Uni *et al.* (1995b).

2.11.3 Passage rate

Passage rate may affect digestibility and absorption of nutrients. In a study over the first 14 days of age, Uni *et al.* (1995b) found passage rate at 4 days of age to be 50% shorter in light strain chicks compared to broiler chicks. However, by day 10 the difference between the strains was not significant. A similar pattern was observed in both strains, with passage time decreasing slightly between days 4 and 7 and increasing thereafter.

2.11.4 Vitelline residue

Nitsan *et al.* (1991b) found that relative to bodyweight, vitelline residue at hatch was greatest for the fast growing Plymouth Rock chickens, and least for the broiler chickens. It was suggested that this is due to the greater demand for nutrients during the embryonic and transition periods of broiler chickens.

2.11.5 Development of digestive organs

Comparison of digestive organ development in broiler chickens and two lines of Plymouth Rock chickens showed similar developmental trends (Nitsan *et al.* 1991b). Relative weights for liver and pancreas showed that organ weights for broiler chickens tended to fall between those of the two lines of Plymouth Rock chickens. Differences in relative liver weights disappeared by day three, with an increase to day six and similar decreases thereafter in all lines. Marked increases in relative pancreas weight were observed in all strains until day nine. This was followed by decreases in all lines, but greater decrease was observed in the broiler chickens. Relative crop weights differed between lines, with the broiler chicken having a heavier crop at hatch, which decreased rapidly to be lighter than the slow growing Plymouth Rock chicken by day nine. Relative proventriculus weight was also highest in the broiler chicken at hatch, but decreased rapidly to be the relatively the lightest of the three lines of chickens by day nine. A similar pattern in relative gizzard weight was observed. Overall the relative size of the food storage organs (crop, proventriculus and gizzard) was largest at hatch, but smallest by day 15 in the broiler chicken strain. Growth pattern of the duodenum, jejunum and ileum was generally similar in all cases, but the relative weights were lowest in the slow growing Plymouth Rock chickens (Nitsan *et al.,* 1991b).

In contrast, Mahagna and Nir (1996) found no age and line interactions between meat and egg type chickens. Throughout the 21 day trial period they observed the relative weight of the digestive tract to be heavier in the broiler chick and the pancreas lighter. The relative liver weight in the broiler chicken was higher than in the egg type chicken and the proventriculus weight lower throughout the trial period.

In a comparative study to 14 days of age, Nir *et al.* (1993) showed a greater increase in relative liver weight in the broiler type chicken than in the egg type chicken, and a slower growing pancreas so that from day eight the relative pancreas weight of the egg type chicken significantly exceeded that of the broiler type chicken. The relative weight of the small intestine was similar between the two types, and only on day eight was the relative weight of the broiler type chicken small intestine greater than that of the egg type chicken.

In summary, all the above studies showed that, compared to the egg type chicken, the broiler chicken showed a faster decline in relative pancreas weight over the first 14 days of age. Furthermore, in contrast to the egg type chicken, the broiler chicken over first few weeks of life may also have relatively smaller food storage organs with which to process a higher relative feed intake.

2.11.6 Gut morphology

Uni *et al.* (1995b) showed that the broiler chickens had larger villi and more enterocytes than the light strain chickens for the first 10 days of age, although the rate of growth of the villi was similar between lines and villus height and perimeter increased in all segments in both lines between 25 and 100% over the period between 4 and 10 days of age. The comparison of line differences at the duodenum, jejunum and ileum showed that the villi are longer and the degree of organisation greater in the heavy strain chicks than in the light strain chicks. As the villi enlarged with age, the number of villi per area decreased in both lines, but the villus volume was consistently greater in the heavy strain chickens. Interestingly, jejunal and ileal villus volume was linearly correlated with feed intake for both strains. The density of enterocytes did not change with age in either line, but it was consistently higher in the heavy chickens. It was noted that the overall length of the small intestine in the light chicken strain was only 70% that of the broiler chicken.

Yamauchi and Isshiki (1991) made similar observations in a comparison of White Leghorn and broiler chickens from 1 to 30 days of age. They observed that at day 1 the duodenal villi of the broiler chicken had many more developed epithelial cell protrusions over the whole apical surface. From 10 days of age, the broiler chicken had villi which were more developed and larger, microvilli which were wider, and from the tip of the duodenal and jejunal villi more active extrusions of epithelial cells. They suggested that the broiler chicken has a greater absorptive surface area, and a more active intestinal function permitting a faster growth rate in the broiler chicken when compared to the White Leghorn birds.

2.11.7 Digestive enzyme activity

2.11.7.1 Pancreatic enzymes

A comparison of pancreatic enzymes in broiler chickens and two lines of Plymouth Rock chickens on days 3, 9 and 15 by Nitsan *et al.* (1991b) found that relative pancreatic trypsin levels at day three for broiler chickens and the fast growing line of Plymouth Rock chickens were two and four times greater than those of the slow growing Plymouth rock chickens, respectively. By day nine, the slow growing line had pancreatic trypsin levels similar to broiler chickens but both were lower than the fast growing Plymouth Rock line. By day 15, broiler chickens had the lowest level of pancreatic trypsin per unit of bodyweight.

Relative pancreatic chymotrypsin levels differed with age. On day three, the fast growing Plymouth Rock line had higher relative levels of pancreatic chymotrypsin than the other two lines. By day nine, the levels were similar for all lines of chickens. However, by day 15, relative pancreatic chymotrypsin levels differed between the three lines with the broiler chickens having the lowest level of pancreatic chymotrypsin per unit of body weight.

Relative levels of pancreatic amylase decreased in all lines over the 15-day trial period. On day three, broiler chickens were intermediate between the two lines of Plymouth

Rock chickens. On day nine, although the pattern remained the same, the differences were no longer significant. On day 15, the broiler chicken and the slow growing line were similar and both had lower amylase levels than the fast growing Plymouth Rock line.

Nir *et al.* (1993) found that up to 14 days of age the specific activity of all the digestive enzymes (units/g) did not differ statistically between broiler chickens and egg type chickens. However the specific activities of amylase, trypsin and chymotrypsin was numerically higher in the egg type chicks than in broiler chicks. When expressed as units of activity per unit of bodyweight, the slightly greater specific activities of amylase, trypsin and chymotrypsin in the egg type chicks were emphasised, becoming statistically significant in some cases.

Uni *et al.* (1995b) compared the production of amylase, trypsin and lipase per gram of feed consumed for light and heavy strain chickens. Daily secretion per gram of feed was higher on day 4 in the heavy chickens. However, daily secretion of trypsin decreased in the heavy strain between days 4 and 7, but not in the light birds. Secretion of amylase and lipase per gram of feed increased only in the light strain birds between days 4 and 7 and decreased in both strains between days 10 and 14. After day 7, no differences were observed between strains.

Nir *et al.* (1993) suggested that although the specific activities (units/g) of pancreatic enzymes were similar between broiler and layer type chicks, the total activities relative to bodyweight indicate that they may be limiting digestion in broiler chicks. During the first week of life, broiler chicks eat a daily food quantity reaching 25 to 30% of bodyweight, an amount double that of egg type chicks. This level of intake is not compensated by increases in the relative size of the pancreas or small intestine, or by the activity of digestive enzymes, which were lower in the broiler chicks. It was concluded that, in comparison to the egg type chicks, the broiler chicks had a similar secretion of digestive enzymes to cope with a higher amount of chyme. In summary, it would appear that the increased feed intake of the broiler chick during week one is not compensated for by a corresponding increase in production of pancreatic digestive enzymes.

2.11.7.2 Brush border enzymes

Mahagna and Nir (1996) measured the activity of saccharase and maltase in broiler and layer type chicks and observed that broiler chicks hatched with a reserve of disaccharidases dramatically exceeding that of the egg type chicks. However, although the activities of both enzymes declined from seven to ten days of age in both types, the decline was much more noticeable in broiler chicks. From day seven to day 21, the enzyme activity in egg type chicks markedly exceeded that in the broiler chicks both in terms of specific activity or activity per unit of bodyweight.

The decline in brush border enzyme activity in the broiler chick may impact upon the digestive capability of the chick over the early period of life.

2.12 Digestion and utilisation of nutrients in newly hatched chicks

2.12.1 Nutrient digestibility

A number of studies have investigated the digestion and absorption of nutrients during the first few weeks post hatch. Noy and Sklan (1995) measured the ileal digestibility of nitrogen, fatty acids and starch over the period of 4 to 21 days of age. Over this period, nitrogen digestibility increased from 78 to 92%. However starch and fatty acid digestibility did not change significantly over this period and ranged from 82 to 89%. Interestingly, this study calculated fat digestibility at four days of age at over 85% in a diet containing 6% added unsaturated fat, and little increase in fat digestion over the remainder of the trial.

Uni *et al.* (1995b) also found ileal nitrogen digestibility increased from 78% on day 4 to close to 90% on day 14. This study found that ileal starch digestibility was between 90 and 95% and changed little over the period of 4 to 14 days of age. Ileal fatty acid digestibility ranged from 80 to 85% over the period and also showed no change between 4 and 14 days of age.

In a series of trials, Batal and Parsons (2002a,b) measured the amino acid digestibility of several diet types in young chicks from hatch to 21 days of age. A trend for digestibility to increase with age was observed in all trials. An exception to this trend was cysteine digestibility where a number of diets showed a numerical decrease in digestibility between days 0-2 and days 3-4. The cysteine digestibility increased again by day 7. With one diet this effect was statistically significant.

Zelenka (1973) reported that the coefficient of apparent digestibility of ether extract decreased from the day of hatch up to eight days of age, and then gradually increased until day 14. Carew *et al.* (1972) fed diets containing 20% fat sourced from either maize oil or tallow and measured fat digestibility during days 2-7 and days 8-15. The average digestibility of the maize oil increased from 85 to 95%, and the beef tallow digestibility increased from 40 to 79% between the two assay periods. Of interest was the trend for the percentage of faecal fat to increase from day 3 to days 5 and 7, before decreasing to day 15. This was attributed to changing transit time by the authors.

A study by Eyssen and Desomer (1963) measured the digestibility of fat over the first 14 days of age in chicks fed diets based on glucose, sucrose, glucose-fructose and starch, without or with virginiamycin. The diets based on glucose, sucrose and glucose-fructose showed a peak of fat malabsorption from the 5th to the 9th days of age during which 30 to 50% of the dietary fat was lost in the excreta. This malabsorption was suppressed by the addition of 100 ppm of virginiamycin. When fed a casein-sucrose diet without antibiotics, a growth depression was observed from 5 to 9 days of age. This was reversed by the addition of procaine penicillin or virginiamycin to the diet. The authors suggest that the growth-stimulating effect of antibiotics may be due to the suppression of gram-positive intestinal bacteria as only antibiotics specifically targeting gram-positive bacteria reduced the malabsorption and stimulated growth. There was little or no malabsorption with the starch diet and no antibiotic response was observed. A further study by Eyssen and Desomer (1967) using gnotobiotic chickens fed a 54% sterilised starch diet found that chicks inoculated with a bacteria free filtrate of faeces and *Streptococcus faecalis* developed malabsorption and growth depression and responded to antibiotics. This occurred over the period of 5 to 9 days of age and by day 14 the affected chicks had a 15% reduction in bodyweight and a 70% increase in the relative weight of the small intestine. The chicks which received both bacteria free filtrate and *Streptococcus faecalis* were more adversely affected than the controls which received either filtrate or *Streptoccus faecalis,* suggesting a growth depressing agent in the faecal filtrate. Although unable to identify the growth depressing agent in the filtrate, the researchers showed that it was inactivated with heat, but not affected by antibiotics and speculated that it may be a virus.

In summary, there is evidence showing that digestibility increases over the first 14 days of life are not linear, and the digestibility of some nutrients may decrease over the period of 5 to 9 days of age before increasing again by day 14. Factors which may cause this short term decrease in digestibility include a rapid increase in feed intake, combined with a decrease in pancreatic enzyme specific activity and a decrease in passage rate. Studies by Eyssen and Desomer (1963; 1967) suggest the changes in gut microflora may significantly reduce fat digestion over this period. By day 14 the more extreme effects of these factors would be diminished as relative feed intake is diminishing, increased pancreas size is producing total increases in pancreatic enzymes, and gut microflora is stabilising.

2.12.2 Energy utilisation.

A series of studies were conducted by Zelenka (1968) to study changes in the ability of broiler chickens to metabolise energy with age. The first study found that the apparent metabolisable energy (AME) of a practical diet decreased rapidly from day 3 after hatching to reach a low point at six to nine days of age before increasing again to 14 days of age. At 14 days of age, the dietary AME was 9.8 percent higher than on day nine. A second study showed a similar pattern of decrease from hatching to a low point at 7-8 days of age followed by a steady increase in AME to 14 days of age. In this study AME was 6.8 percent higher at day 14 than on day eight. Murakami *et al.* (1992) also found that metabolisability of dietary energy and absorption of dietary lipids were highest at hatch, then declining to their lowest at day 5 or 6 and gradually increasing thereafter.

A series of trials were conducted by Batal and Parsons to measure changes in AME of maize-soy diets over the first 14 days of age of broiler chicks. The first study (Batal and Parsons, 2002a) showed a linear increase in AME from day 2 to day 14, with nitrogen corrected AME (AME_n) values of 12.43 MJ/kg on day two increasing to 14.35 MJ/kg on day 14. A second study (Batal and Parsons, 2002a) reported AME_n of 13.23 MJ/kg on days 0-2, which decreased to 12.55 MJ/kg on days 3-4 before increasing again to 13.52 MJ/kg on day 14. The third study (Batal and Parsons, 2002b) observed that

AME_n values were higher at 0-2 days of age (12.67 MJ/kg) than at 3-4 days of age (11.41 MJ/kg), and then increased again by day 14 (13.49 MJ/kg).

Corless and Sell (1999) reported an increase in AME between days 4 and 7 in turkey poults. However the day 4 values were 23% below the calculated values for the diets. They also recorded a drop in AME between days 7 and 14, which was attributed to a coronavirus infection.

In summary, there is evidence to suggest a decline in metabolisable energy values over the first week of life, followed by an increase in the metabolisable energy of diets up to 14 days of age. This is primarily due to the decrease in fat digestibility observed by Zalenka (1973) and Eyssen and Desomer (1963)

2.13 Nutritive value of cereals for poultry

It is known that the nutritional value of grains differs as a result of the specific composition of the grain. Wheat tends to have a lower metabolisable energy value than maize or sorghum, partially as the result of a higher digesta viscosity caused by the level of soluble non starch polysaccharides (NSP) in the grain which reduces digestion and absorption of a range of nutrients. However there are large variations in the NSP level of cereals, particularly of wheats, and therefore a wide variability in AME values has been reported for different wheat samples (Choct *et al.*, 1999). Other factors affecting the metabolisable energy value of grains include the proportion of starch in the grain (Pirgozliev *et al.*, 2003) and starch digestibility (Nicol *et al.*, 1993). A confounding factor in determining poultry AME values is a wide bird variation which is more pronounced in poorer quality wheat (Wiseman *et al.*, 1994) and sex differences (Hughes, 2001). Variation in grain characteristics may be due to variety, seasonal effects, growth sites, crop treatment, fumigants, post-harvest storage conditions and length of storage (Hughes and Choct, 1999).

2.13.1 Cereal energy values

The metabolisable energy value for maize is somewhat higher than that of wheat (~14 versus ~12.5 MJ/kg on an as fed basis) although there is wide variation within each cereal type. One reason for the difference between cereals is the lower level of NSP in maize. Maize typically contains only 1 g/kg water soluble NSP compared with 24, 45 and 46 g/kg for wheat, barley and rye, respectively (Cowieson, 2005). Maize also tends to have a higher level of starch (>600 g/kg) than wheat, which coupled with the lower level of NSP, enhances the metabolizable energy value of the grain. However the factors affecting the AME and nutritional value of the cereals are still not well understood. For example, Thacker (2005) compared wheat and maize based diets in broiler chickens and reported that the wheat based diets had higher digestibility coefficients for dry matter and energy than the maize based diets.

2.13.2 Digestive tract development

High fibre cereals generally increase the size of the gastrointestinal tract. Jorgensen *et al.* (1996) found that diets supplemented with pea fibre, oat bran or wheat bran increased both the length and weight of the gastrointestinal tract with increasing dietary fibre level. The weight and size of the caeca increased more than other segments of the gastrointestinal tract. Fibre level increased gut fill by 0.17g/kg bodyweight for each gram of NSP given as pea fibre. Steenfeldt (2001) observed a significant positive correlation between arabinoxylan level and the relative weights of the duodenum, jejunum and ileum and a negative correlation between relative ileal weight and both AME and fat digestibility in broiler chickens fed diets based on wheat.

2.13.3 Gut morphology

A study by Iji (1999) found that guar gum and xanthin gum significantly increased crypt depth in both the jejunum and ileum. Villus height was increased in the ileum but reduced in the jejunum. The reduction in villus height in the jejunum was accompanied by a reduction in both cell population and cell size. Carre *et al.* (2005) compared hard and soft wheats and found no significant effect on gut morphology. This lack of significance was attributed to high individual variability.

2.13.4 Anti-nutritive activity of cereal non-starch polysaccharides

There is a large body of evidence to show that soluble NSPs are a significant cause of the variation in the nutritional value of cereals. Choct and Annison (1990) found that the AME of rice, maize, sorghum, wheat, triticale, barley and rye were highly correlated with the summed levels of pentosans and β-glucans found in the cereals. Annison (1993) makes a case for soluble NSP having anti-nutritive activity and being largely responsible for the low AME wheat phenomenon. He puts forward the following reasons in support of this hypothesis:

1. Wheat AME values are negatively correlated with soluble non-starch polysaccharide levels,

2. Low level addition (30g/kg) of commercially available non-starch polysaccharide depresses the AME of the diet,

3. Degradation of the cell wall polysaccharides *in situ* by addition of glycanases to broiler diets raises AME values, and

4. Addition of purified wheat arabinoxylan to broiler diets depresses the AME in a dose-dependent manner.

Annison (1991) also showed a highly significant negative correlation (r = -0.91, P<0.0001) between AME values and total NSP in thirteen wheat samples. The addition of wheat pentosans to a commercial type broiler diet caused a general inhibition of nutrient digestion affecting starch, fat and protein (Choct and Annison, 1990).

In contrast, Nicol *et al.* (1993) found no correlation between AME and total NSP content, or AME and arabinoxylan content in 10 samples of wheat of varying AME content, although they found a high correlation between AME and arabinose/xylose ratio, which is an indication of branching. Wootton *et al.* (1995) compared 19 Australian wheats and 12 American wheats, and reported a positive correlation between AME and pentosan level in Australian wheats. Choct *et al.* (1999) suggest that this finding may be due to the comparatively narrow range of AME values into which the 19 samples fell (16 samples exceeded 14 MJ/kg DM and none were below 13 MJ/kg DM). Phillips *et al.* (2004) found that dietary NSP content was not a consistent predictor of viscosity in the ileum, and furthermore, that the addition of xylanase did not significantly reduce ileal viscosity. Although the mechanisms underlying the anti-nutritive effects of NSP on wheat quality are not completely clear, the fact that poor quality wheats respond to the addition of exogenous xylanase points to arabinoxylans (NSP) having an important role in determining on the nutritional value of the grain.

2.13.5 Physical and chemical properties of soluble NSP which may contribute to anti-nutritive properties

2.13.5.1 Viscosity

When dissolved in water, most polysaccharides give viscous solutions. The viscosity is dependent on a number of factors, which include the size of the molecule, whether it is branched or linear, the presence of charged groups, the surrounding matrix, and the concentration of the polysaccharides (Smits and Annison, 1996) . At low concentrations, the NSP increases viscosity by directly interacting with the water molecules. As the concentration increases, the NSP molecules begin to interact and become entangled which increases the viscosity further. Eventually the interactions can reach a level where gel formation takes place (Annison and Choct, 1994, Smits and Annison, 1996). Increases in digesta viscosity may inhibit nutrient digestion by impeding the diffusion of digestive enzymes and their substrates and products (Annison 1993) or through viscosity-microflora interaction. Morgan and Bedford (1995) showed a good correlation between viscosity and AME ($R^2 = 0.645$ $P = 0.009$) in wheat. In contrast Steenfeldt (2001) found no correlation between intestinal viscosity and nitrogen corrected AME or fat digestibility.

2.13.5.2 Surface activity

Polysaccharides can present charged surfaces, which may associate with food surfaces or lipid micelles. For example, pancreatic lipases act only at the lipid/water interface. It is possible that the presence of NSP at this interface may be the mechanism by which they inhibit the digestion of fats (Annison 1993).

2.13.5.3 Water holding capacity

Soluble polysaccharides can trap water through the formation of networks. This gives rise to the viscous or gel effect referred to above (Annison and Choct, 1994; Smits and Annison, 1996).

2.13.5.4 Viscosity-microflora interaction

Hubener *et al.* (2002) reported that dietary cereals producing high intestinal viscosities lead to increased overall bacterial activity in the small intestine. Furthermore, he reported that compared to a maize diet, colony forming units of mucosa associated bacteria such as enterobacteria and enterococci were higher in a wheat/rye diet. Annison (1993) suggested that interaction between gut microflora and NSP causes variability in AME in young chickens and may be partly responsible for the low-AME wheat phenomenon. Choct *et al.* (1996) demonstrated that where large amounts of soluble NSP were present in the diet there was an increase in small intestine fermentation which negatively impacted on chick performance. Langhout *et al.* (2000) added highly methylated citrus pectin to a maize-based diet, which was fed to both conventional and germ-free chicks. They reported that faecal digestibilities of organic matter, crude fat, starch, and amino acids were reduced in the conventional chicks but not in the germ free chicks. Smits and Annison (1996) reported that caecectomised chickens show a smaller negative effect from wheat pentosans than intact chickens on the digestion of long chain fatty acids. Furthermore, the addition of antibiotics to rye or wheat based diets improves chick growth and feed utilization, and the improvement is always greater in rye based diets than in wheat based diets, suggesting that the anti-nutritive activity of NSP is related to the gut microflora of the chicken (Annison and Choct, 1991).

2.13.5.5 Increase in the secretion of endogenous protein

Annison (1993) suggested that the presence of arabinoxylans may increase the secretion of endogenous protein as well as inhibiting the degradation of protein in the gastro-intestinal tract. Low (1989) reported that the effects of NSP on the gastro-intestinal tract of the pig increased the secretory output of the salivary glands, stomach, liver pancreas and intestinal wall. Angkanaporn *et al.* (1994) suggest that a reduction in apparent amino acid digestion observed with pentosan dietary supplementation is due to endogenous amino acid losses at low levels of pentosan inclusion, and direct inhibition of protein breakdown and amino acid absorption with high levels of pentosan inclusion.

2.13.6 Summary

In summary, the anti-nutritive effect found in viscous grains, such as wheat, is a complex phenomenon. The means by which nutrient digestibility is reduced remains poorly understood. However it is generally believed that the presence of soluble NSP has a significant role in the metabolisable energy and feed value of the cereals. Further research should investigate the role of underlying biochemical and physical events including development of gut microflora and the relationship between gut development, enzyme secretion, and nutrient digestibility in wheat diets. The literature suggests that complex interactions between chick and feed substrate are involved in the development of digestive capability and an understanding of these is necessary to optimise performance in the modern broiler chicken.

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Chapter 3

Performance, Digestive Tract Development and Gut Morphology of the Newly Hatched Broiler Chick Fed Diets Based on Wheat, Sorghum or Maize

3.1 ABSTRACT

Diets based on wheat, sorghum and maize and formulated to contain iso-energy and iso-lysine levels were fed to broiler chickens from days 1-14 post hatch. Birds fed the maize based diet grew faster ($P < 0.05$) than those fed the sorghum based diet. Weight gains of birds fed the wheat based diet did not differ ($P > 0.05$) from those fed either maize or sorghum based diets. There were numerical differences in feed:gain with the sorghum based diet having the highest feed per gain, but the differences were not significant ($P > 0.05$).

The relative weights of digestive organs and digestive tract showed no significant cereal treatment differences ($P > 0.05$). The treatments had no effect ($P > 0.05$) on the relative length of the digestive tract or gut morphology measurements. The wheat based diet contained a commercial xylanase which may have ameliorated the digesta viscosity of this diet and reduced the effect of NSP on gut structure and morphology. Furthermore, gut morphology samples showed a high degree of variation, suggesting that a large sample size would be necessary before significant differences could be determined.

3.2 INTRODUCTION

Differences in growth rate and feed efficiency of broiler chickens are known to result from diets based on different grain types (Ao and Choct, 2004). Cereals contain varying levels of non-starch polysaccharides (NSP) giving rise to differing viscosity levels in both the feeds manufactured from these cereals and in the digesta of chickens fed these feeds. Increased digesta viscosity is known to reduce digestion and absorption of a range of nutrients (Annison, 1993). Wheat based diets are more viscous than maize based diets as they contain higher levels of NSP and as a result tend to be less efficiently utilised than maize based diets by growing birds. Another factor which may influence cereal performance is the starch content of the grain. The starch content of both wheat and maize varies widely, which may contribute to the differences in the efficiency of growth (Choct *et al.*, 1999; Cowieson, 2005).

The gastrointestinal tract is a major consumer of nutrients in animals, with rapid cell turnover utilising significant quantities of both nutrients and energy. An increase in the proportional size of the gastrointestinal tract will therefore reduce feed efficiency. High NSP cereals may increase the size of the gastrointestinal tract. Jorgensen *et al.* (1996) found that diets supplemented with wheat bran increased both the length and weight of the gastrointestinal tract. The weight and size of the caeca increased more than other segments of the gastrointestinal tract. Steenfeldt (2001) observed a significant positive correlation between arabinoxylan level and the relative weights of the duodenum, jejunum and ileum.

Greater crypt depth indicates increased villus cell proliferation, and therefore an increase in nutrients being utilised by the gastrointestinal tract. Iji (1999) found that guar gum and xanthin gum significantly increased crypt depth of both the jejunum and ileum, suggesting that NSP may promote gastrointestinal tract cell turnover. Nutrients are absorbed through the villus epithelium and changes in villus height alter the area available for nutrient absorption. Non-starch polysaccharides in guar gum and xanthin gum increased villus height in the ileum but reduced villus height in the jejunum. The reduction in villus height in the jejunum was accompanied by a reduction in both cell population and cell size.

The digestive system of the newly hatched chick is not fully developed and this impacts on the efficiency with which the chick utilises dietary nutrients. Dietary differences would therefore be expected to be more pronounced during the immediate post-hatch period.

Performance differences in chicks fed diets based on wheat, sorghum or maize were measured in this study and the hypothesis that these differences in performance are related, partly, to differences in relative digestive tract size and intestinal morphology was tested.

3.3 MATERIALS AND METHODS

All experimental procedures were approved by the Massey University Animal Ethics Committee (Anon, 2003) and complied with the Massey University Code of Ethical Conduct for the Use of Live Animals for Teaching and Research (2003).

3.3.1 Diets

Three dietary treatments based on wheat, sorghum and maize were used in the trial. The diets were formulated to have similar levels of AME, amino acids, calcium and available phosphorus. The diets were formulated to meet or exceed the NRC (1994) requirements for all nutrients. The wheat-based diet was supplemented with a commercial xylanase as this is standard commercial practice and necessary if the research results are to have practical relevance. All diets were steam pelleted at 60 ^{0}C. The ingredient composition of the diets is shown in Table 1.

3.3.2 Experimental Procedures

Day-old male broilers (Ross 308) were obtained from a commercial hatchery (Tegel Foods Ltd, Auckland), weighed and allocated to 18 cages of eight chicks each. Each dietary treatment was then assigned to six replicate cages. The chicks were housed in 3-tier electrically heated brooder pens (Plate 1) with 400cm^2/bird space allocation. Brooding temperature was maintained at 32 ^{0}C on day 1 decreasing to 27 ^{0}C on day 14. Ventilation was controlled by a central ceiling extraction fan and wall inlet ducts. Feed and water were available at all times. Bodyweight and feed intake were measured at weekly intervals. Moisture loss from the feed was measured over the first seven days and feed intake adjusted. Mortality was recorded on a daily basis. The trial lasted for 14 days.

Table 1: Composition and calculated analysis (g/100g) of diets based on wheat, sorghum and maize

Ingredient	Wheat diet	Sorghum diet	Maize diet
Wheat	65.76	-	-
Sorghum	-	61.08	-
Maize	-	-	58.62
Soybean meal	27.34	32.19	35.18
Vegetable oil	2.73	2.09	1.78
Dicalcium phosphate	2.03	1.91	2.17
Limestone	0.34	0.94	0.78
Salt	0.14	0.16	0.23
Lysine.HCl	0.34	0.29	0.18
DL-methionine	0.24	0.33	0.25
L-threonine	0.12	0.08	0.03
Sodium bicarbonate	0.36	0.33	0.18
Trace mineral premix	0.25	0.25	0.25
Vitamin premix	0.05	0.05	0.05
Xylanase[1]	+	-	-
Calculated analysis,			
AME, MJ/kg	12.5	12.5	12.5
Crude protein	22.0	22.0	22.0
Lysine	1.35	1.35	1.35
Methionine + cystine	0.95	0.95	0.95
Calcium	0.95	0.95	0.95
Available phosphorus	0.48	0.48	0.48

[1] Kemzyme, Kemin Industries, Singapore, added 0.075 g/100g diet.

Plate 1. Battery brooder unit.

3.3.3 Collection and Processing of Samples

On day 14, two birds per replicate pen were weighed and killed by intravenous injection of Pentobarbitone 300 (National Veterinary Supplies Ltd, East Tamaki, Auckland) and the gastrointestinal tract (GIT) removed. The lengths of the intestinal segments (duodenum, jejunum, ileum) and caeca were obtained. The weights of the liver and pancreas, and empty weights of proventriculus, gizzard, duodenum, jejunum, ileum and caecum were recorded (Plate 2).

Two more birds per replicate were euthanased and tissue samples were obtained for gut morphological measurements. The gut samples were taken from four sites: site 1: proximal duodenum (the top of the descending portion of the duodenal loop), site 2: duodenal loop (the base of the duodenal loop), site 3; distal duodenum/proximal jejunum (at the top of the ascending portion of the duodenal loop) and site 4: distal jejunum/proximal ileum (around Meckel's diverticulum) (Plate 3). The samples were immediately fixed in Bouin's fluid for a 24 hour period and then stored in a 70% alcohol solution until further processing.

3.3.4 Morphological Measurement

Gut morphology examinations were carried out according to the method described by Iji *et al.* (2001) with minor modifications. Five villi per slide were measured. Gut samples were fixed in Bouin's solution and then stored in a 70% alcohol solution before being paraffin-embedded. Each sample was sectioned at a thickness of 7 µm, stained with alcian blue/haematoxylin-eosin and examined by light microscopy. The slides were viewed on a Zeiss Axiophot microscope. Visual measurements of villus height, crypt depth and epithelium thickness were made at 100X magnification using imaging software (Image Pro Plus, Version 4.1.0.9, Media Cybernetics, Silver Spring, MD, USA) (Plate 4).

3.3.5 Data Analysis

The data were analysed by simple ANOVA using the General Linear Model procedure of the SAS® (SAS Institute, 1997) with pen means as the experimental unit for performance data. Morphological measurements were nested within the chicken. Differences were determined by LSD and considered significant at $P<0.05$.

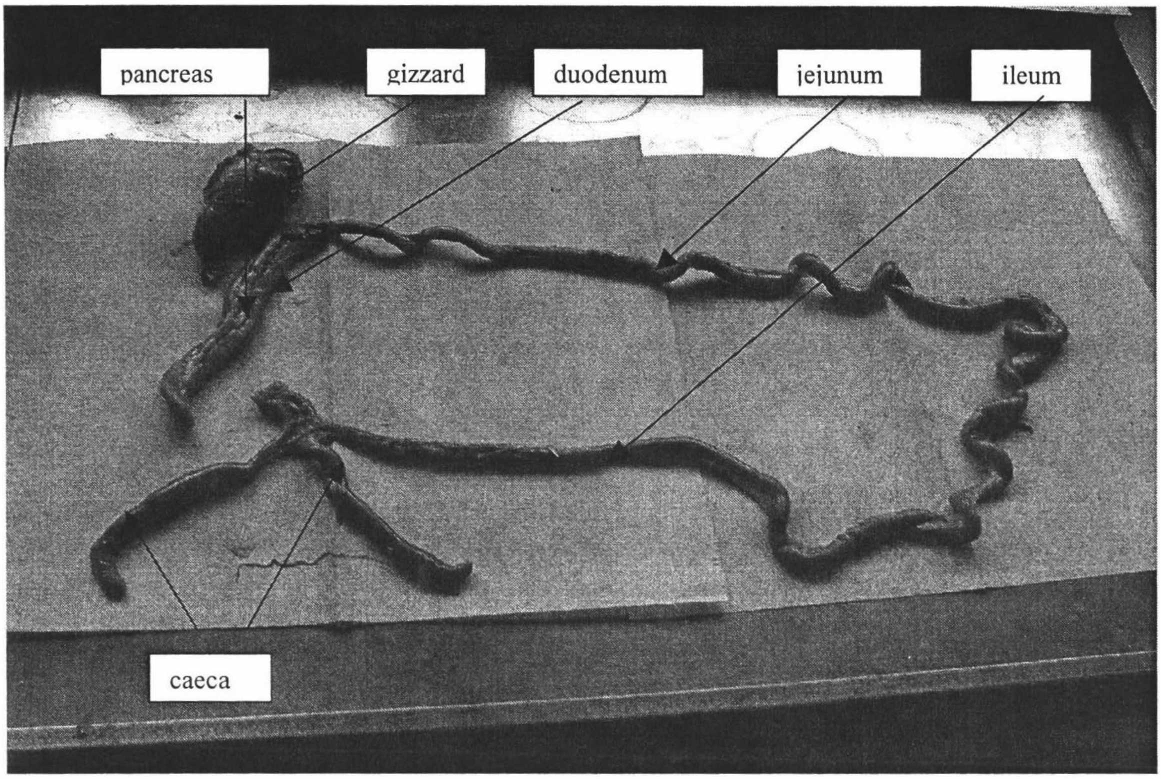


Plate 2. Gastro-intestinal tract measurements.

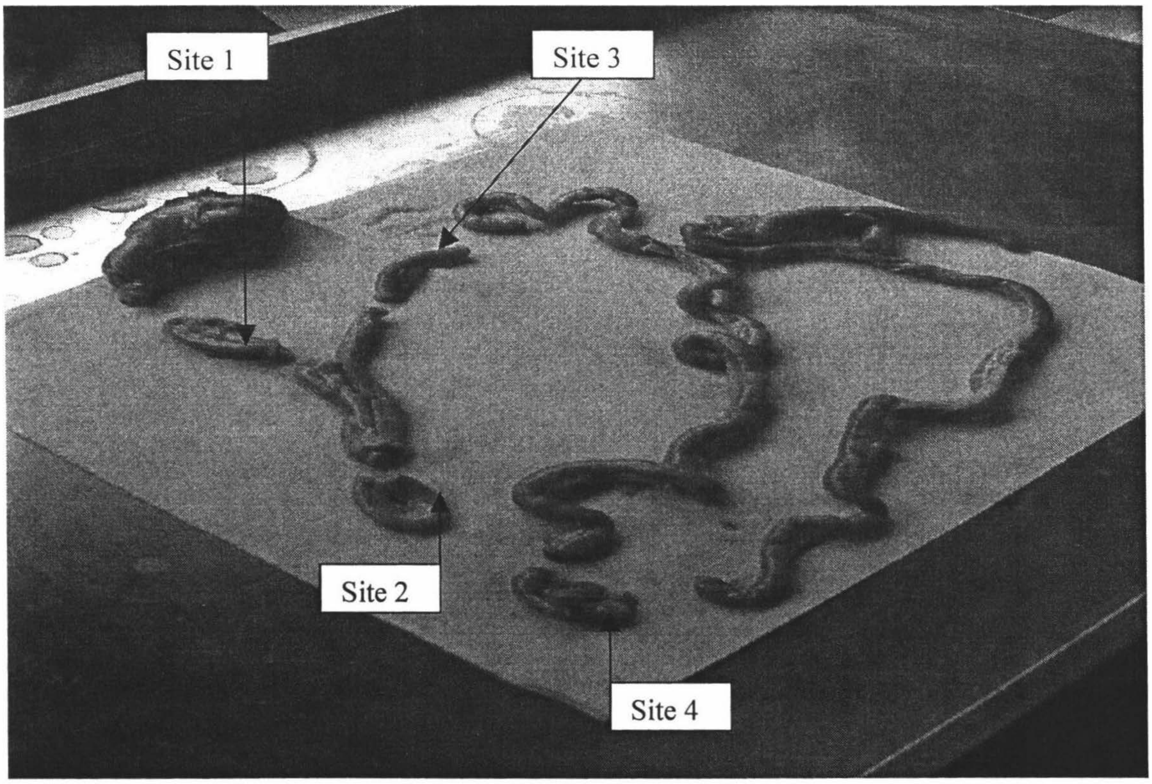


Plate 3. Sample sites for gut morphology measurements.

3.4 RESULTS

3.4.1 Performance data

The performance data are shown in Table 2. Birds fed the maize based diet grew faster ($P < 0.05$) than those fed the sorghum based diet. Growth rate for the wheat based diet did not differ ($P > 0.05$) from either maize or sorghum. There was no difference ($P > 0.05$) in feed intake between the three dietary treatments, although there was a numerical trend for feed intake to increase from sorghum to wheat to maize diet, which paralleled weight gain of the birds. There was a numerical improvement in feed per gain from sorghum to wheat to maize, but the differences were not significant ($P > 0.05$). The patterns in performance over the two week trial period were similar to those for the first week.

Table 2: Weight gain, feed intake and feed per gain ratio of male broiler chicks fed diets based on wheat, sorghum or maize[1]

		Wheat	Sorghum	Maize	Pooled SEM
1-7 days	Weight gain, g/bird	142^{ab}	131^{b}	148^{a}	3.8
	Feed intake, g/bird	154	151	160	5.1
	Feed per gain g/g	1.108	1.155	1.093	0.030
1-14 days	Weight gain, g/bird	438^{ab}	422^{b}	463^{a}	10.8
	Feed intake, g/bird	568	553	588	13.6
	Feed per gain, g/g	1.304	1.324	1.277	0.025

[a,b] Means in the same row with different superscripts are significantly different ($P<0.05$).
[1] Mean of six replicates (8 birds/ replicate).

3.4.2 Digestive tract measurements

The relative weights of digestive organs and segments of gastrointestinal tract are shown in Table 3. There were no treatment differences ($P >0.05$) for any of the parameters, but there was a tendency for birds fed sorghum based diets to have a larger gizzard ($P = 0.09$) and ileum ($P = 0.07$).

Table 3: Relative day 14 digestive organ and tract weights (g/100g body weight) of male broiler chicks fed diets based on wheat, sorghum or maize

	Wheat	Sorghum	Maize	SEM
Relative weights[1]	3.39	3.25	3.23	0.14
Liver				
Pancreas	0.38	0.34	0.34	0.02
Proventriculus	0.63	0.67	0.62	0.04
Gizzard[2]	1.64	1.87	1.69	0.07
Duodenum	1.02	1.02	0.99	0.05
Jejunum	2.06	1.94	2.09	0.07
Ileum[3]	1.65	1.72	1.50	0.07
Small intestine[4]	4.73	4.68	4.57	0.16
Caeca	0.38	0.42	0.37	0.02

[1] Mean of six replicates (2 birds/ replicate).

[2] P=0.09.

[3] P=0.07.

[4] Duodenum + jejunum + ileum.

The relative lengths of the digestive tract segments are shown in Table 4. The lengths of the duodenum, jejunum, ileum and caeca and small intestine showed no treatment differences ($P > 0.05$) although birds fed the maize based diet tended ($P = 0.07$) to have shorter caeca.

Table 4: Relative day 14 lengths of digestive tract segments (cm/100g BW) of male broiler chicks fed diets based on wheat, sorghum or maize

	Wheat	Sorghum	Maize	SEM
Relative lengths[1]	4.55	4.43	4.31	0.14
Duodenum				
Jejunum	12.18	12.15	12.17	0.40
Ileum	11.12	11.69	11.10	0.35
Small intestine[2]	27.85	28.27	27.58	0.79
Caeca[3]	2.20	2.25	1.95	0.08

[1] Mean of six replicates (2 birds/ replicate).

[2] Duodenum + jejunum + ileum.

[3] P=0.07.

3.4.3 Morphological measurements

The influence of dietary treatments on the morphological measurements at the four sampling sites (Plate 3) is summarised in Table 5. No significant treatment differences ($P > 0.05$) were observed at any of the four sites measured. All morphological measurements showed a high degree of variation, suggesting that a larger sample size would be necessary for any significant differences to be detected. The only significant effect was the effect of sampling sites on villous height ($P < 0.01$). The villous height increased from proximal duodenum to duodenal loop and then decreased to distal duodenum and distal jejunum (Table 5 and Plate 4). These effects were consistent for all three diets.

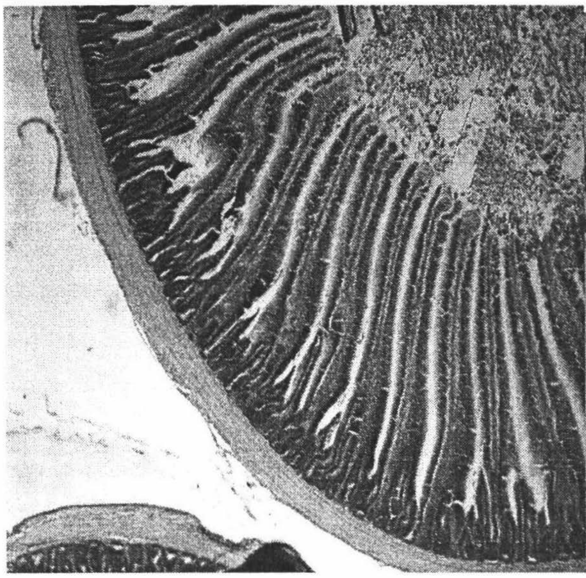
Table 5: Intestinal morphological measurements of day 14 male broiler chicks fed diets based on wheat, sorghum and maize [1,2]

	Wheat	Sorghum	Maize	Pooled SEM
Proximal duodenum (μm)				
Villous tip – crypt base	1530	1478	1526	225
Villous height	1303	1241	1296	200
Crypt depth	211	197	201	53
Epithelial thickness	41	38	37	7.6
Duodenal loop (μm)				
Villous tip – crypt base	1791	1793	1795	262
Villous height	1562	1556	1572	231
Crypt depth	215	209	211	50
Epithelial thickness	44	43	42	8.1
Duodenum/jejunum (μm)				
Villous tip – crypt base	1075	1069	1114	175
Villous height	879	876	902	154
Crypt depth	189	196	198	42
Epithelial thickness	38	40	44	9.3
Jenunum/ileum (μm)				
Villous tip – crypt base	810	723	812	130
Villous height	542	586	660	108
Crypt depth	149	138	150	34
Epithelial thickness	33	31	34	4.7

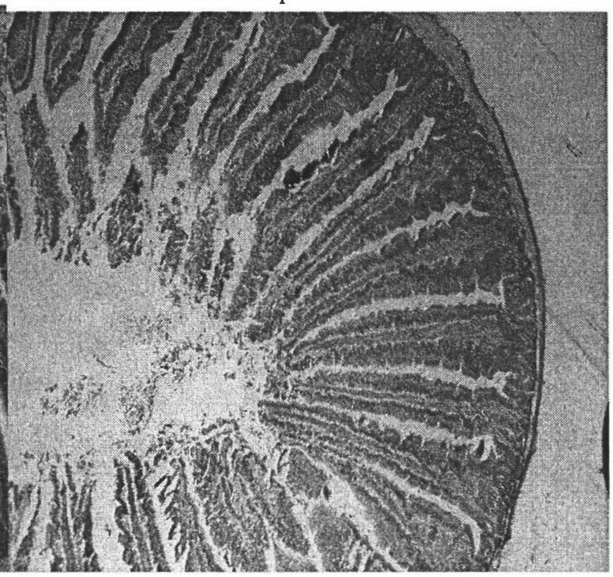
[1] Mean of 12 observations.

[2] Differences were not statistically significant ($P > 0.05$).

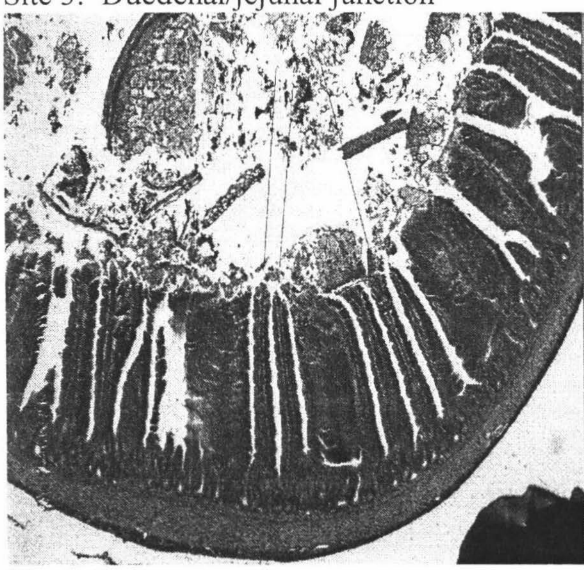
Site 1: Proximal duodenum



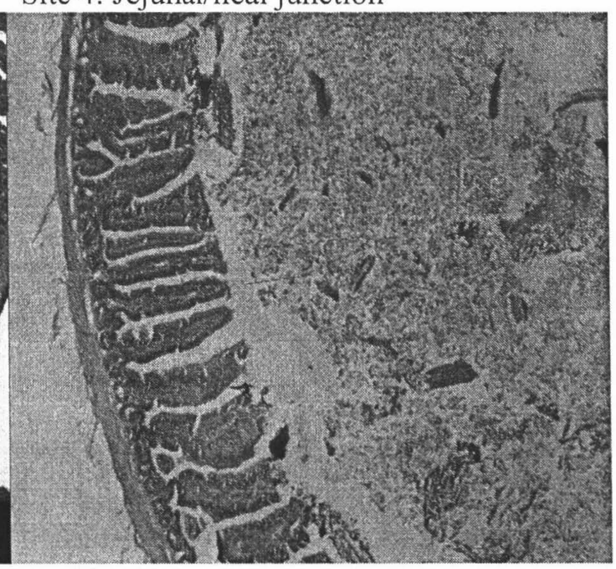
Site 2: Duodenal loop



Site 3: Duedenal/jejunal junction



Site 4: Jejunal/ileal junction



Site 3: Site 4:

Plate 4. Cross section of the gut (100 X magnification) at the four sampling sites in birds fed the wheat based diet

3.5 DISCUSSION

Based on the reported differences in NSP levels in the three cereals (Choct, 1997), it was expected that chicks fed the maize and sorghum based diets would grow faster and more efficiently than those fed the wheat based diet, however this may be confounded by the inclusion of xylanase in the wheat diet. The dietary effects on digestive tract size and gut morphology were considered as they may contribute to variation in bird performance.

Cereals contain varying levels of NSP giving rise to differing viscosity levels in both the diets and in the digesta of chickens (Choct, 1997). Non-starch polysaccharide levels in wheat are higher and more variable than those of maize, giving rise to a high variability in chicken performance. This is attributed, in part, to increases in digesta viscosity in wheat based diets which may lower nutrient digestion by impeding the diffusion of digestive enzymes and their substrates and products (Annison, 1993) or through viscosity-microflora interactions (Hubener *et al.*, 2002). Non-starch polysaccharides have also been shown to increase the size of organs of the gastrointestinal tract (Jorgensen *et al.*, 1996) and to affect gut morphology (Iji, 1999) both of which may reduce the efficiency of feed utilisation.

Results from the current trial showed the chicks fed the maize based diet grew faster and had a numerically lower feed per gain than those fed the sorghum based diet. The performance of birds fed the wheat based diet was not significantly different from either the maize or sorghum based diets. This result was unexpected, but may be explained by the fact that the wheat based diet contained a commercial xylanase which may have reduced the digesta viscosity effects and improved the nutrient availability. Comparing bird performance of birds fed wheat, sorghum and maize based diets, Ao and Choct (2004) found that sorghum and maize fed birds grew faster and had better feed efficiency than those fed a wheat based diet and that maize fed chickens grew more efficiently than sorghum fed chickens. However, in their study, exogenous xylanase was not added to the wheat based diet.

Tulasi *et al.* (2004) found that substituting sorghum for maize gave a similar growth rate, but a higher feed per gain in the chicks fed the sorghum based diets However, Connor *et al.* (1976) compared the apparent metabolisable energy (AME) of sorghum and maize hybrids and found significant differences in AME for location and year of harvest, and significant hybrid differences for sorghum. Black *et al.* (2005) reported that available energy is utilised less efficiently in sorghum than in wheat possibly because sorghum protein has a lower digestibility than wheat protein. Another possible reason for the lower performance of the sorghum diet may be the presence of tannins, which have anti-nutritional effects including reduced protein digestibility and poor feed efficiency (Nyachoti *et al.*, 1997).

Dietary levels of NSP have been shown to influence the size of the gut with a higher NSP level increasing both the length and weight of the gastrointestinal tract, and the relative weights of the duodenum, jejunum, ileum and caeca (Jorgensen *et al.,* 1996; Steenfeldt, 2001). In the present study, cereal base had no effects on the relative weights or lengths of the digestive tract. As noted earlier, the addition of xylanase may explain the lack of effects in the wheat based diet.

Non-starch polysaccharides have been observed to affect gut morphology. A study by Iji (1999) found that NSPs in guar gum and xanthin gum significantly increased crypt depth in both the jejunum and ileum. Villus height was increased in the ileum but reduced in the jejunum. Carre *et al.* (2005), comparing hard and soft wheats, however, found no significant effect of NSP on gut morphology, which was attributed to high individual variability. Similarly, a wide individual variability was observed between birds in the current trial and this variability may be responsible, in part, for the lack of significant differences for any of the gut morphological measurements.

In conclusion, contrary to the original hypothesis, feeding of diets based on wheat, a grain with high NSP levels, resulted in similar chick performance to those fed on diets based on maize and sorghum, which have lower levels of NSP. This unexpected result is possibly due to the addition of exogenous xylanase, which may have ameliorated the effects of NSP on digesta viscosity. Nevertheless, in agreement with published data (Annison and Choct, 1991; Bedford and Schulze, 1998), these results suggest the use of exogenous xylanases in wheat based diets may be beneficial. Performance differences were seen between maize and sorghum diets, but these differences were not related to digestive tract or gut morphology.

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Chapter 4

Nutrient Utilisation of Diets Based on Wheat, Sorghum or Maize by the Newly Hatched Broiler Chick

4.1 ABSTRACT

Two experiments were conducted to measure changes in nutrient utilisation in the newly hatched chick. The first experiment determined the nitrogen corrected apparent metabolizable energy (AME_n) of diets based on wheat, sorghum and maize during the first two weeks post-hatch in broiler chickens. Changes in the total tract digestibility of starch and fat were also measured. The second experiment was conducted to confirm the results of Experiment 1 using wheat and maize based diets and was of 21 days duration.

In both experiments, changes to AME_n with age were similar for all diets, declining from day 3 to days 5-9, and increasing again. In experiment 1, cereal effects were significant ($P < 0.05$) with maize and sorghum based diets having higher AME_n values than the wheat based diet. In experiment 2, cereal effects were significant ($P < 0.05$) with the maize based diet having a higher AME_n than the wheat based diet.

In experiment 1, total tract starch digestibility determined for days 5, 7 and 14 showed no cereal differences ($P > 0.05$). Age effects were significant ($P < 0.05$) with starch digestibility declining from day 5 to day 7, and then increasing again. Total tract fat digestibility on day 7 was significantly lower for the wheat and sorghum based diets than for the maize based diet, but no cereal differences ($P > 0.05$) were observed on days 5 or 14. Age effect was highly significant with fat digestibility declining from day 5 to day 7 and increasing again. These results showed that nutrient utilisation is compromised during the first week of life of the broiler chick.

4.2 INTRODUCTION

With the first 14 days post-hatch now representing 40% of the total life of the broiler chicken, an understanding of the development of digestive capability of the newly hatched chick is of increasing importance. Despite this, published data on the changes with age in the energy and nutrient utilisation in the young broiler chick are limited (Zelenka, 1968; Murakami et al., 1982; Batal and Parsons, 2002a). Furthermore, some of these studies have been conducted decades ago and the genetic makeup of broilers has changed significantly over this period, particularly during the past decade. To assess the significance of developmental changes in nutrient utilisation in the newly hatched broiler chick, it is necessary to reassess these earlier data in light of genetic changes in broiler chickens. In general, the studies undertaken by Zelenka (1968) and Batal and Parsons (2002a) are suggestive of changes in the utilisation of dietary nutrients over the first 14 days of age in the broiler chicken. In general, both studies showed nitrogen-corrected apparent metabolisable energy (AME_n) to be high immediately after hatching, followed by a decline over the next several days and an increase from day 7 onwards.

Two studies, which were undertaken to determine changes in the digestive capability of the modern broiler chicken during the post-hatch period, are reported in this chapter. The first experiment determined the changes in AME_n and the total tract digestibility of starch and fat of diets based on wheat, sorghum or maize over the first 14 days of age. The second experiment was conducted to confirm the results of the first study and examined the changes in AME_n of diets based on wheat or maize over the first 21 days post-hatch.

4.3 MATERIALS AND METHODS

The experimental procedures were approved by the Massey University Animal Ethics Committee (Anon, 2003) and complied with the Massey University Code of Ethical Conduct for the Use of Live Animals for Teaching and Research (2003).

4.3.1 Experimental Procedures

The management of birds, housing, composition of diets and design used in Experiment 1 are described in Chapter 3. Experiment 2 was conducted to further confirm the results for Experiment 1. The assay procedures in Experiment 2 were similar to those in Experiment 1, except that only the diets based on wheat or maize were used, collection periods were 48 hours and the experiment was conducted for 21 days.

4.3.2 Collection and Processing of Samples

Experiment 1

On days 3, 5, 7, 9 and 14, feed intake was accurately measured and total excreta collection carried out over a 24-hour period. A sample of feed was taken daily to measure any moisture loss, and this was used to correct feed intake. Excreta were accurately weighed and mixed in a blender to provide a homogenous slurry from which two representative samples were obtained per pen. Excreta samples were then freeze dried. Diet and dried excreta samples were ground to pass through a 1.0 mm sieve and stored in airtight plastic containers until chemical analysis. Dry matter (DM), gross energy (GE), nitrogen and titanium oxide content were determined in all diets and excreta. Starch and fat were determined for the three diets and excreta samples from days 5, 7 and 14.

Experiment 2

Total excreta collection was undertaken over a 48-hour period on days 1, 3, 5, 7, 9, 13 and 20. The feed and excreta samples were processed using the procedure outlined in experiment 1, and the dry matter, nitrogen and gross energy (GE) were determined.

4.3.3 Chemical Analysis

Dry matter content was determined using AOAC (1994) procedure (AOAC 930.15). Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid. Starch was measured using an assay kit (Megazyme, Boronia, VIC, Australia) based on the use of thermostable-α-amylase and amyloglucosidase (McCleary *et al.*, 1997). Titanium was measured on a UV spectrophotometer following the method of Short *et al.* (1996). Crude fat was determined as petroleum spirit extractable material (40-60 ^{0}C) using soxhlet extraction.

Nitrogen content was determined by the Dumas method (Sweeney, 1989) using a CNS-2000 carbon, nitrogen and sulphur analyser (LECO® Corporation, St. Joseph, Michigan, USA).

4.3.4 Calculations

The AME values in Experiment 1 were calculated by both total collection and by marker ratio methods, while AME in Experiment 2 was calculated only by the total collection method. The following formulas were used, with appropriate corrections for differences in DM content.

Total collection method

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

Marker method

$$\text{AME (MJ/kg)} = [(\text{GE/ Ti})_{diet} - (\text{GE/ Ti})_{excreta}] / (\text{GE/ Ti})_{diet} \text{ x GE}_{diet}$$

where $(\text{GE/ Ti})_{diet}$ = ratio of GE to titanium in the diet and

$(\text{GE/ Ti})_{excreta}$ = ratio of GE to titanium in the excreta.

Nitrogen-corrected AME values were calculated using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958) using the formula.

$$\text{AME}_n \text{ (MJ/kg)} = (\text{AME} - ((36.52 * \text{N retention})/1000))$$

where N retention = g/kg DM intake

Total tract nutrient digestibility

Excreta nutrient digestibility coefficients (DC) were calculated in experiment 1 on days 5, 7 and 14 by the marker method using the following formula.

$$\text{DC} = [(\text{Nutrient/ Ti})_{diet} - (\text{Nutrient/ Ti})_{excreta}] / (\text{Nutrient/ Ti})_{diet}$$

4.3.5 Data Analysis

The data were subjected to repeated measures analysis using the General Linear Model of SAS® (SAS Institute, 1997) to assess cereal differences over age and cereal x age interactions. Differences were consid bered significant at $P < 0.05$, although probability values up to $P \leq 0.10$ are shown in the text if the data suggest a trend. When a significant F-test was detected, means were separated using the least significant difference test.

4.4 RESULTS

4.4.1 Experiment 1

4.4.1.1 Apparent Metabolisable Energy

Total collection method

The AME_n of the diets as measured by total collection is shown in Table 1. Cereal differences were significant ($P < 0.05$) on days 3, 5, 7 and 9 but not on day 14. On days 5, 7 and 9, wheat had a lower ($P < 0.05$) AME_n than sorghum or maize, but the AME_n of sorghum and maize were similar ($P > 0.05$). Age effects were significant ($P < 0.001$) with AME_n values decreasing from day 3 to day 7 and then increasing to day 14. Cereal x age interaction was significant ($P < 0.05$) with differences between cereals being greater on days 7 and 9 (Figure 1).

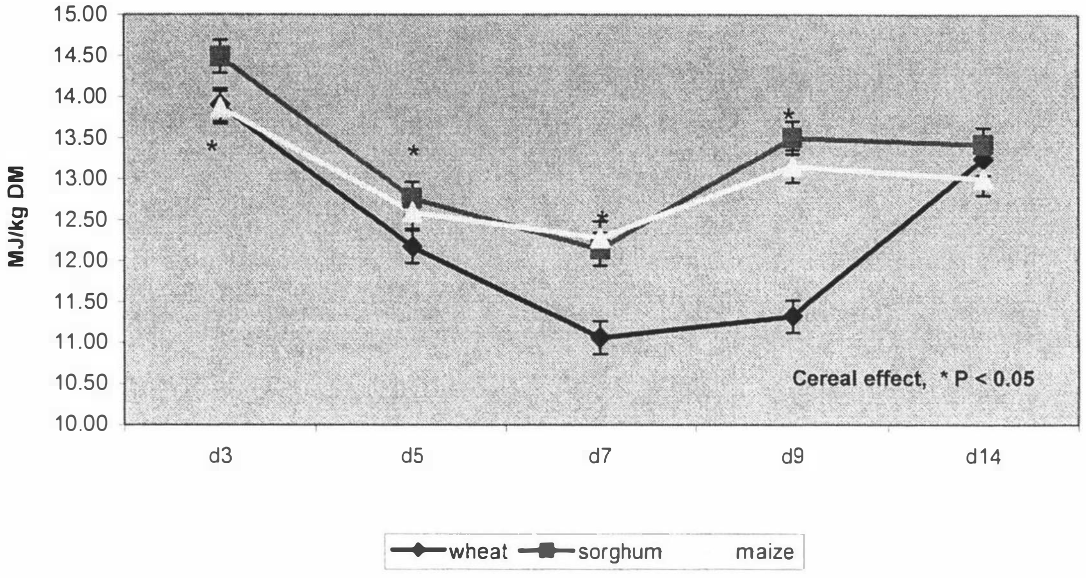
Table 1. Least square means for AME_n (MJ/ kg DM) of wheat, sorghum and maize-based diets for broiler chickens during the first 14 days post hatch - calculated by the total collection method

	Day					Pooled
Cereal	**3**	**5**	**7**	**9**	**14**	**SEM**
Wheat	13.90^{a4}	12.17^{a2}	11.06^{a1}	11.32^{a1}	13.24^{3}	0.20
Sorghum	14.49^{b4}	12.76^{b2}	12.14^{b1}	13.50^{b3}	13.42^{3}	
Maize	13.87^{a3}	12.59^{ab12}	12.28^{b1}	13.15^{b2}	13.00^{2}	

^{a,b} Different superscript letters within a column are statistically different ($P < 0.05$) LSD.

^{1,2,3} Different superscript numbers within a row are statistically different ($P < 0.05$) LSD.

Figure 1. AME$_n$ values for wheat, sorghum and maize based diets for broiler chickens - calculated by the total collection method



Marker method

The AME$_n$ of the diets, calculated using the marker ratio, is shown in Table 2. The AME$_n$ values determined by this method followed a somewhat similar trend to those determined by the total collection method in terms of cereal and age effect. Cereal differences were significant ($P < 0.05$) on days 5, 7, 9 and 14. Age effect was significant ($P < 0.001$) with AME$_n$ values decreasing from day 3 to 5-9 days of age, and then increasing again to day 14. Cereal x age interaction was significant ($P < 0.05$) due to a greater AME$_n$ reduction on day 7 for wheat and sorghum than for maize (Figure 2).

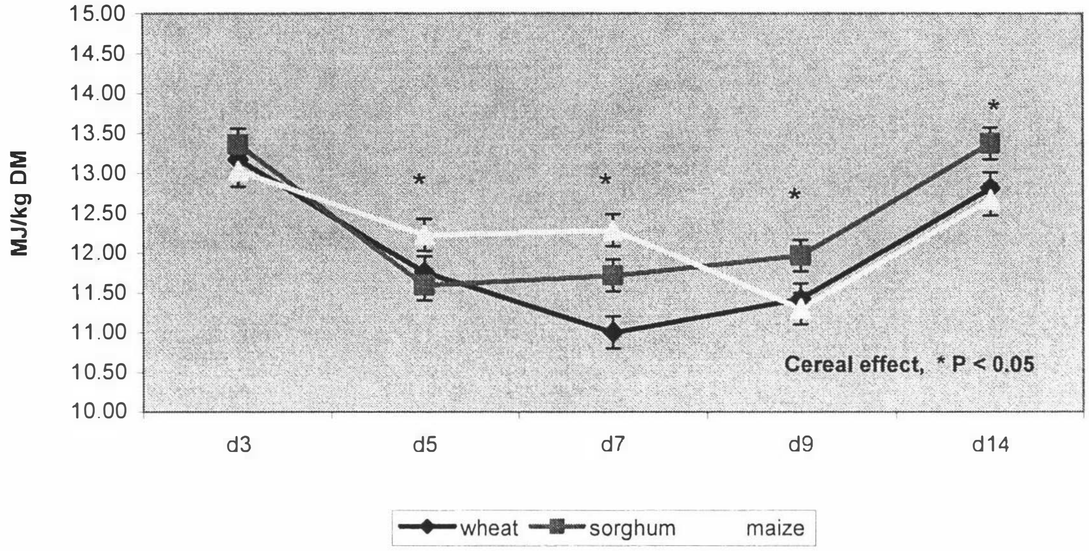
Table 2. Least square means for AME (MJ/ kg DM) of wheat, sorghum and maize-based diets during the first 14 days post hatch - calculated by the titanium marker method

	Day					Pooled
Cereal	**3**	**5**	**7**	**9**	**14**	**SEM**
Wheat	13.17^{3}	11.76^{ab2}	11.00^{a1}	11.42^{ab12}	12.82^{ab3}	0.20
Sorghum	13.36^{2}	11.60^{a1}	11.72^{b1}	11.97^{b1}	13.38^{b2}	
Maize	13.03^{3}	12.23^{b2}	12.29^{c2}	11.30^{a1}	12.68^{a23}	

^{a,b} Different superscript letters within columns are statistically different ($P < 0.05$).

^{1,2,3} Different superscript numbers within rows are statistically different ($P < 0.05$).

Figure 2. AME_n for wheat, sorghum and maize based diets – calculated by the titanium marker method



4.4.1.2 Total tract starch digestibility

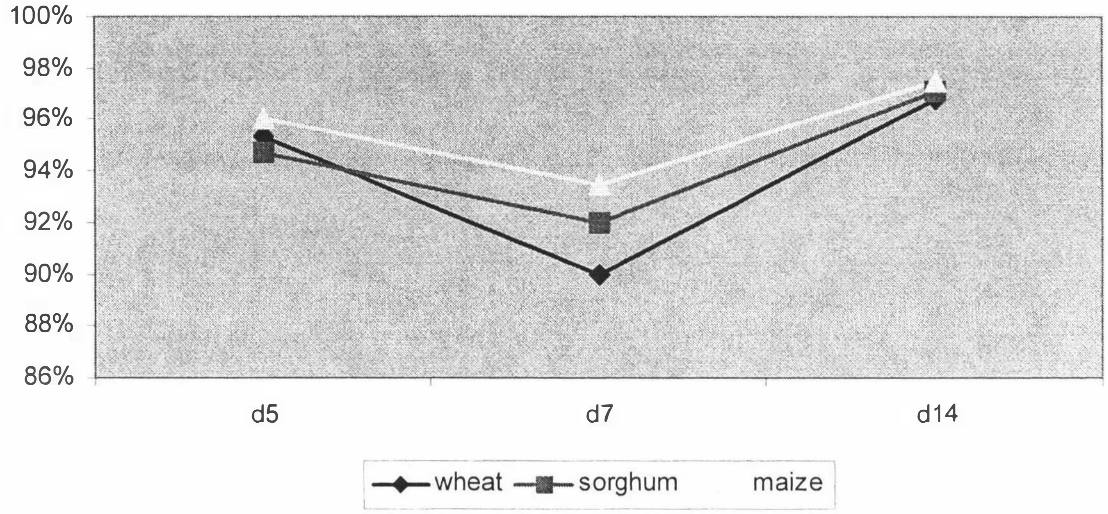
The influence of cereal base and age on the total tract starch digestibility is shown in Table 3. Cereal effects were not significant ($P > 0.05$), but significant ($P < 0.001$) age effects were observed. Starch digestibility on days 5 and 14 was similar between cereals, but differed on day 7 with maize having the highest digestibility and wheat the lowest. These differences resulted in a trend ($P = 0.06$) for cereal x age interaction. Starch digestibility declined from day 5 to day 7 and then increased again to day 14 (Figure 3).

Table 3. Total tract starch digestibility coefficients for broilers fed wheat, sorghum and maize based diets at 5, 7 and 14 days of age

	Day			Pooled
Cereal	**5**	**7**	**14**	**SEM**
Wheat	0.95^{2}	0.90^{1}	0.97^{2}	0.006
Sorghum	0.95^{2}	0.92^{1}	0.97^{3}	
Maize	0.96^{2}	0.94^{1}	0.98^{2}	

[1,2,3] Different superscripts within a row are statistically different ($P < 0.05$).

Figure 3. Starch digestibility coefficients for broilers fed wheat, sorghum and maize based diets at 5, 7 and 14 days of age



4.4.1.3 Total tract fat digestibility

The influence of cereal base and age on total tract fat digestibility is shown in table 4. Cereal differences were significant on day 7, with wheat and sorghum fat digestibility being lower ($P < 0.05$) than maize fat digestibility. Age differences were significant ($P < 0.001$). Cereal x age interaction was not statistically significant ($P > 0.05$). Fat digestibility declined from day 5 to day 7, and then increased again to day 14 (Figure 4).

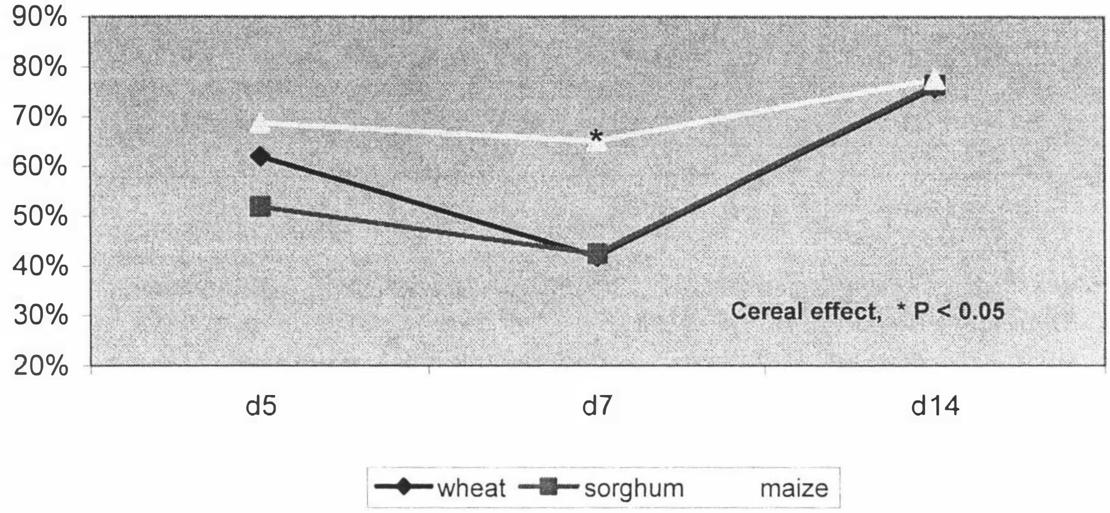
Table 4. Total tract fat digestibility coefficients for broilers fed wheat, sorghum and maize based diets at 5, 7 and 14 days of age

	Days			Pooled
Cereal	**5**	**7**	**14**	**SEM**
Wheat	0.62^{2}	0.42^{a1}	0.76^{3}	0.048
Sorghum	0.52^{1}	0.43^{a1}	0.76^{2}	
Maize	0.69	0.65^{b}	0.78	

[a,b] Different superscript letters within a column are statistically different ($P < 0.05$).

[1,2,3] Different superscript numbers within a row are statistically different ($P < 0.05$).

Figure 4. Fat digestibility coefficients for broilers fed wheat, sorghum and maize based diets at 5, 7 and 14 days of age



4.4.1.4 Nitrogen retention

The influence of cereal base and age on nitrogen retention is shown in Table 5 and Figure 5. Cereal effects on nitrogen retention were significant ($P < 0.01$) with the sorghum diet generally to having a lower nitrogen retention than the wheat or maize based diets. Age effects were significant ($P < 0.001$). The highest level of nitrogen retention was on day 3, before reducing to the lowest point at day 7 with no further increase. There was no cereal x age interaction ($P > 0.05$).

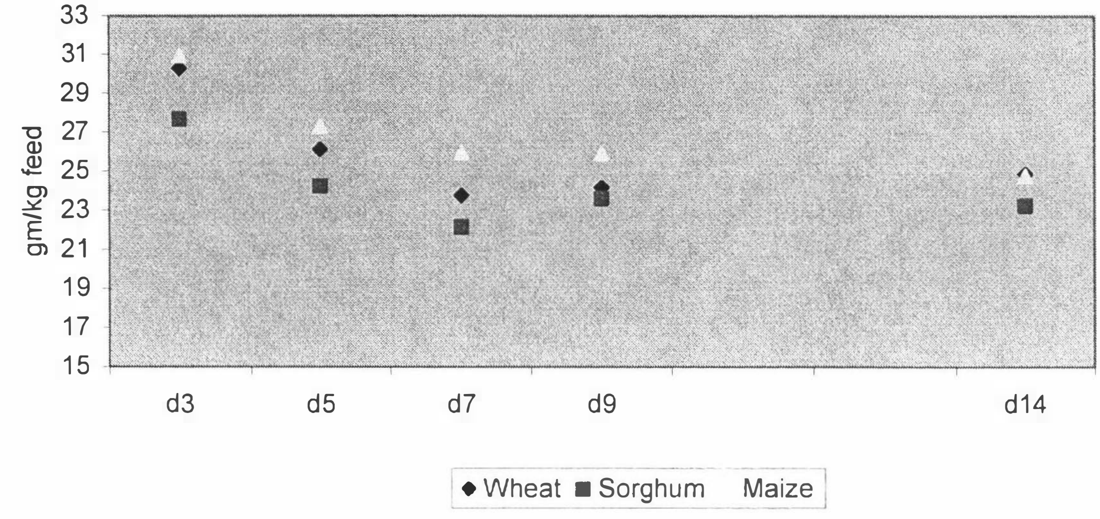
Table 5. Nitrogen retention (g/ kg DM intake) for broilers fed wheat, sorghum and maize based diets at 5, 7 and 14 days of age

	Day					Pooled
Cereal	**3**	**5**	**7**	**9**	**14**	**SEM**
Wheat	30.3^{a3}	26.1^{a2}	23.7^{b1}	24.1^{a1}	24.9^{a12}	0.53
Sorghum	27.7^{b3}	24.2^{b2}	22.2^{b1}	23.6^{a12}	23.3^{b12}	
Maize	30.9^{a3}	27.3^{a2}	26.0^{a12}	25.9^{b12}	24.8^{a1}	

a,b Different superscript letters within columns are statistically different ($P<0.05$).

1,2,3 Different superscript numbers within rows are statistically different ($P<0.05$).

Figure 5. Nitrogen retention (g/kg DM intake) for broilers fed wheat, sorghum and maize based diets at 5, 7 and 14 days of age



4.4.1.5 Feed intake

Feed intake data over the 14-day trial period are summarised in Table 6. Feed intake increased linearly ($P < 0.001$), but no differences ($P > 0.05$) between cereals were observed.

Table 6. Changes in daily feed intake (g DM/day)

	Day					Pooled
Cereal	**3**	**5**	**7**	**9**	**14**	**SEM**
Wheat	14^{1}	24^{2}	32^{3}	40^{4}	69^{5}	1.30
Sorghum	14^{1}	24^{2}	31^{3}	41^{4}	68^{5}	
Maize	14^{1}	25^{2}	31^{3}	42^{4}	69^{5}	

^{1,2,3,4,5} Different superscripts within a row are statistically different ($P < 0.001$).

4.4.2 Experiment 2

4.4.2.1 Apparent metabolisable energy

The changes in AME_n values of wheat and maize based diets during 1-21 days post-hatch are shown in Table 7. Cereal and age effects were significant ($P < 0.01$) and there was no cereal x age interaction ($P > 0.05$). The AME_n of maize based diets was greater ($P < 0.05$) than those of wheat based diets at all ages. AME_n values decreased from day 2 to a low point between days 6-10 and then increased to day 14 (Figure 6). There was no further increase in AME_n between day 14 and day 21.

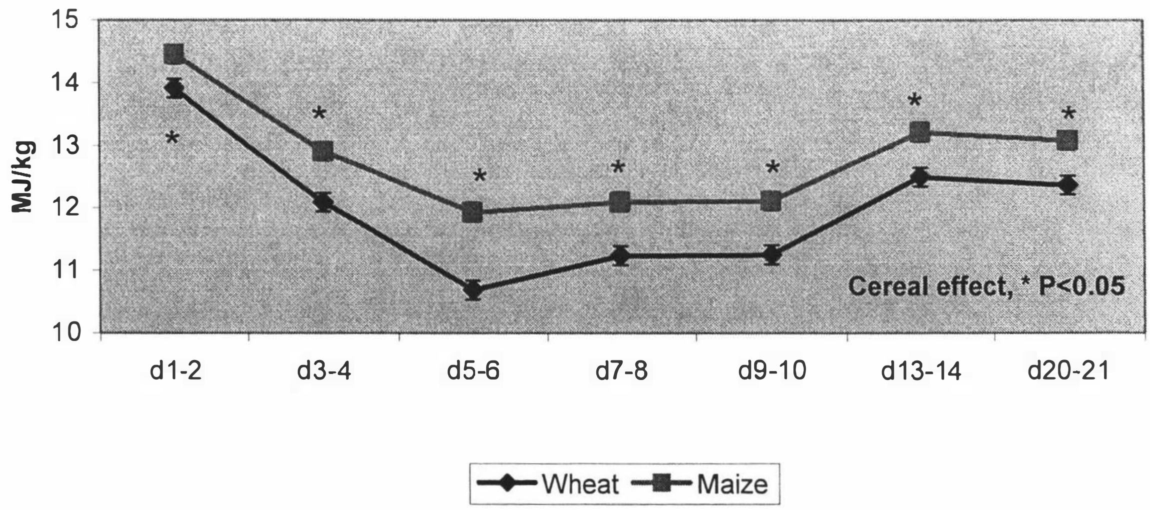
Table 7. Least square means for AME (MJ/ kg DM) of wheat and maize-based diets for the first 21 days post hatch

	Day							Pooled
Cereal	**2**	**4**	**6**	**8**	**10**	**14**	**21**	**SEM**
Wheat	13.91^{a4}	12.09^{a3}	10.68^{a1}	11.24^{a2}	11.25^{a2}	12.49^{a3}	12.35^{a3}	0.15
Maize	14.46^{b3}	12.91^{b2}	11.93^{b1}	12.09^{b1}	12.11^{b1}	13.21^{b2}	13.08^{b2}	

^{a,b} Different superscript letters within a column are statistically different (P<0.05).

^{1,2,3} Different superscript numbers within a row are statistically different (P<0.05).

Figure 6. Changes in AME_n values for wheat and maize based diets during 1-21 days post hatch



4.4.2.2 Nitrogen retention

The changes in nitrogen retention coefficients of wheat and maize based diets during 1-21 days post-hatch are shown in Table 8 and Figure 7. Cereal ($P < 0.05$) and age ($P < 0.001$) effects on nitrogen retention were significant. The nitrogen retention was highest on day 2, before reducing to the lowest point at day 10 before slightly increasing by day 14. There was no cereal x age interaction ($P > 0.05$).

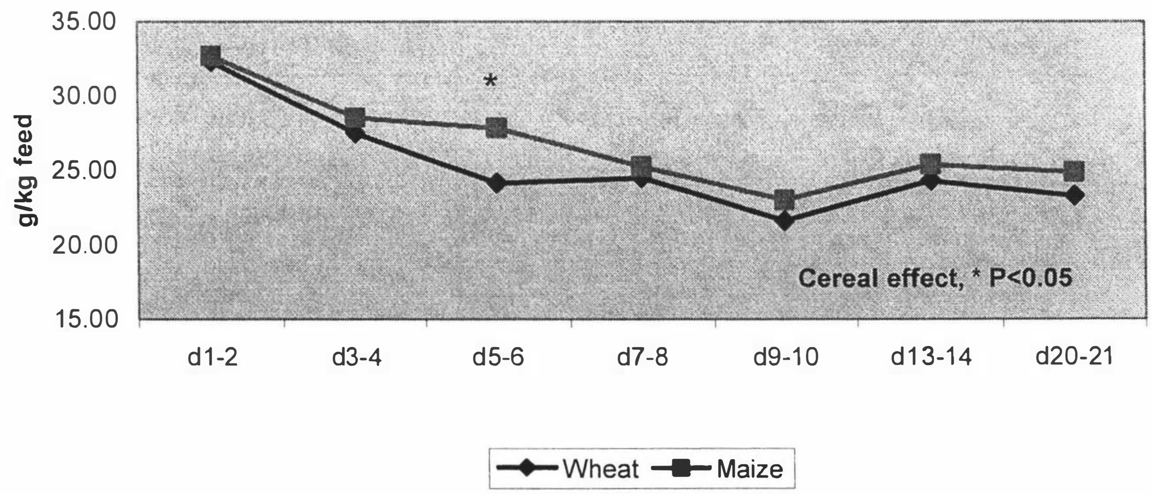
Table 8. Nitrogen retention (g/kg DM intake) of wheat and maize-based diets for the first 21 days post hatch

	Day							Pooled
Cereal	**2**	**4**	**6**	**8**	**10**	**14**	**21**	**SEM**
Wheat	32.31^{4}	27.51^{3}	24.16^{a2}	24.49^{2}	21.64^{1}	24.31^{2}	23.31^{2}	0.59
Maize	32.69^{4}	28.56^{3}	27.84^{b3}	25.26^{2}	23.01^{1}	25.40^{2}	24.88^{2}	

^{a,b} Different superscript letters within columns are statistically different (P<0.05).

^{1,2,3} Different superscript numbers within rows are statistically different (P<0.05).

Figure 7. Nitrogen retention (g/kg DM intake) of wheat and maize-based diets for the first 21 days post hatch



4.4.2.3 Feed intake

Feed intake increased ($P < 0.001$) with advancing age (Table 9). Feed intake of the two diets was different ($P < 0.05$) at all ages from day 5 onwards.

Table 9. Daily feed intake (g DM/ bird), 1-21 days post-hatching

	Day							Pooled
Cereal	**1-2**	**3-4**	**5-6**	**7-8**	**9-10**	**13-14**	**20-21**	**SEM**
Wheat	9.02	15.29	22.92^{b}	29.70^{b}	36.32^{b}	60.70^{b}	95.39^{b}	0.89
Maize	8.85	14.57	19.52^{a}	23.96^{a}	30.89^{a}	56.18^{a}	89.13^{a}	

^{a,b} Different superscript letters within a column are statistically different ($P<0.05$).

4.4.2.4 Feed intake per unit of bodyweight

The bodyweights were recorded on days 7, 14 and 21, and feed intake per kilogram of bodyweight calculated. Feed intake per kilogram of bodyweight declined with advancing age as shown in Table 10.

Table 10 Feed intake per unit of bodyweight (g/kg body weight)

	Days		
Cereal	**7**	**14**	**21**
Wheat	210	150	120
Maize	180	150	110

4.5 DISCUSSION

Viscous grains with relatively high levels of non-starch polysaccharides (NSP) such as wheat are known to have lower AME than non-viscous grains such as maize and sorghum which contain low levels of NSP (Hughes and Choct, 1999) and the anti-nutritive effects of NSP may be greater in young birds. Thus the choice of cereal base in pre-starter diets may be critical during feed formulation to maximise nutrient availability and bird performance. The current studies were undertaken to evaluate the changes in the AME_n of diets based on wheat, sorghum or maize when offered to young broiler chicks. In both experiments, significant differences were observed in AME_n between cereals, with the wheat based diet having a lower AME_n than those of maize and sorghum based diets. These results are consistent with expected results (Hughes and

Choct, 1999) and suggest that the use of non-viscous cereals in pre-starter diets will be beneficial.

In all diets, AME_n was observed to decrease from day 3 to days 5-9 and then to increase thereafter. These effects were more pronounced in the wheat based diet. The results are consistent with those reported by Zelenka (1968) who observed a rapid decline in the ability of chicks to metabolise the energy in practical diets between hatch and 7-9 days of age, and a subsequent increase by day 14. Later studies (Batal and Parsons, 2002a; Murakami *et al.*, 1992) also showed a similar decline in energy utilisation over the first few days of age. In contrast, Batal and Parsons (2004) determined the AME of a number of diet formulations and found that the AME generally increased over the first 14 days of age for most diets. However, a decrease an AME_n at 3-4 days of age was observed in some diets and the changes in AME with age were not always consistent.

Studies investigating the changes in nutrient digestion with age have generally focussed from Day 7 onwards. It is generally believed that starch digestion appears not limiting in young chicks (Moran, 1982). In the present study, total tract digestibility of starch in all three diets was determined to reach values of 95% by day 5 post-hatching. These results are consistent with previous reports (Uni *et al.*, 1995b; Noy and Sklan, 1997) with total tract starch digestibility of over 95% by 4-10 days post-hatching. However, the modifying effects of caecal microflora on nutrient digestion is now well documented (Ravindran *et al.*, 1999) and nutrients that disappear post-ileum are not considered beneficial to chickens (Ravindran and Bryden, 1999). In future studies, nutrient digestibility must be measured at the terminal ileum rather than in the excreta. Current evidence suggests that ileal digestibility of starch from wheat can be as low as 51% even in 3-week-old broilers (Svihus and Hetland, 2001).

Studies have shown that digestion of both unsaturated and saturated fats is low in young chicks (Carew *et al.*, 1972; Polin and Hussein, 1982; Sell *et al.*, 1986) and this poor digestibility has been attributed to low lipase activity or to insufficient bile secretion. The results from the present study also confirm that fat digestibility is low in the young chick. Total tract digestibility of fat, mainly from soybean oil, was low (52 to 69%) at day 5 in all three diets and declined to around 43% by day 7, except in the maize based diet. By day 14, the digestibility increased to over 76%. Fat digestibility estimates determined in the current study during week 1 post-hatching are considerably lower than the value of 86% determined in 2 to 7 day-old chicks by Carew *et al.* (1972).

The changes in AME_n with age in the present study closely paralleled trends for total tract fat digestibility. Similar decreases in fat digestibility over the first week post-hatch age have been previously reported (Eyssen and Desomer, 1963; Mateos *et al.*, 2002; Zelenka, 1973). A recent study by Gracia *et al.* (2003) showed a decline in AME from day 4 to day 8 followed by an increase on day 15 which appeared to be associated with a decrease in organic matter, nitrogen and ether extract retention on day 8. In contrast, Batal and Parsons (2002) found no significant decrease in either fat or starch digestibility with age.

The causes for the decrease in energy utilisation and nutrient digestion observed during the first week of life of the newly hatched chick are unclear, however there may be a number of contributing factors.

1. Yolk sac. Over the first few days of life, the yolk sac contributes to the overall nutritional intake of the chick and has a beneficial effect on the function and development of the digestive tract (Zelenka, 1968; Murikami *et al.,* 1992). The initial drop in digestibility may reflect the decreasing beneficial effect of the yolk sac, and the subsequent rise in digestibility may be reflective of the developing digestive capability in the neonatal chick.
2. Changes in microflora. The gut of the newly hatched chick is sterile and is rapidly colonised by microflora after hatching. Changes in microflora continue in the immediate post-hatch period before stabilising around three weeks of age (McBurney *et al.*, 2003). Developing gut microflora may contain significant proportions of bacterial species which lower nutrient digestion by competing for nutrients or by negatively impacting on the gut environment or gut mucosal layer. Eyssen and Desomer (1963) reported that the fat malabsorption in the chicks during 5-9 days of age was eliminated through the feeding of antibiotics which target gram positive bacteria.
3. Digestive enzyme availability. In a comparison of egg-type and broiler chickens, it has been shown that the increased feed intake of the broiler chicken is not compensated by an increase in the size of pancreas or in disaccharidase activity at the intestinal brush border (Mahagna and Nir, 1996). Furthermore, a

significant decrease was reported in maltase and saccharase activity per unit of bodyweight in the broiler chick over the first 7 days of age. The activity levels of specific pancreatic enzymes were measured by Nitsan *et al.* (1991) who reported that the specific activities of pancreatic trypsin, amylase and lipase decreased during the first 3 to 6 days after hatching and then increased. These results suggest that, during the first week of life, levels and/or activity of pancreatic enzymes may limit nutrient digestibility. Iji *et al.* (2001b) concluded that the level of pancreatic enzyme activity is a constraint to digestion and growth in the young broiler chick.

4. Digesta mixing. Over the first few days of life, relative gut size has been shown to markedly increase. In relation to body weight, the weight of small intestine peaks at 7-14 days of age and then rapidly decreases (Iji *et al.*, 2001a; Ravindran *et al.*, 2006). Consistent with these changes in small intestinal capacity, feed intake per unit of bodyweight declines with age (Table 10; Nir *et al.*, 1993; Uni *et al.*, 1995a). It is tempting to speculate that the very rapid growth in gut size and feed intake may lead to some short term mechanical inefficiency in the transit and/or mixing of digesta and influence nutrient digestion.

In conclusion, the present data demonstrate that nutrient digestion is compromised during the first week of life of the broiler chick. Nutrient utilisation, as measured by AME_n was high at day 3, and rapidly declined to days 5-9 before increasing again. Similar age related effects were seen in total tract fat and starch digestibility. A number of contributory factors have been suggested, but further work is required to identify the specific causes of the decline in digestibility during the early stages of chick development.

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Chapter 5

Total Tract Retention of Minerals in Diets Based on Wheat, Sorghum or Maize When Fed to the Newly Hatched Broiler Chick

5.1 ABSTRACT

Samples of diets and excreta from Experiment 1, described in Chapter 4, were analysed for minerals to determine changes in the apparent total tract mineral retention of the broiler chick during the first two weeks post-hatch when fed diets based on wheat, sorghum or maize. The diet and excreta samples were analysed for calcium, phosphorus, potassium, sodium, magnesium, iron, manganese, zinc and copper, and their retention was determined. The retention coefficients of individual minerals differed widely and the retentions of major minerals were much greater than those of minor minerals. The cereal effect was significant ($P < 0.05$) for several minerals, with a general tendency for the sorghum diet to have greater retention than maize or wheat diets. Age effects were significant ($P < 0.05$) for all minerals. In general mineral retention coefficients were higher at day 3, declined to day 7 and remained unchanged to day 14. Decline in mineral retention with age was similar in all three diets.

5.2 INTRODUCTION

Published data on the changes with age in mineral absorption and utilisation in the young broiler chick are limited (Ravindran, 2004; Ravindran *et al.*, 2006). Coefficients of mineral absorption for poultry are in some cases derived from values published for human infant liquid formulas (Ahmad and Sarwar, 2006). Accurate data on mineral retention by the young chicks are necessary if diets are to meet the requirements of the chick during the early stage of development. Furthermore, increasing awareness of the environmental cost of waste disposal is placing more emphasis on minimising excessive dietary mineral levels. To assess the significance of developmental changes in mineral utilisation in the newly hatched broiler chicken it is necessary to more fully understand mineral digestion and retention. Accurate data on mineral digestibility and retention needs to be obtained through faecal measurement as it is probable that the action of the large intestine affects retention of certain minerals, particularly sodium. Ravindran *et al.* (2006) determined negative ileal digestibility for sodium in 21 day old chickens.

This study was undertaken to determine changes in the mineral retention of the modern broiler chicken during the post-hatch period, and measurements were undertaken over the first two weeks post-hatch.

5.3 MATERIALS AND METHODS

5.3.1 Experimental Procedures and Processing of Samples

The management of birds, housing, composition of diets and design are described in Chapter 3. A classical total excreta collection was carried out on days 3, 5, 7, 9 and 14. Details of sample processing are described in Experiment 1 of Chapter 4.

5.3.2 Chemical Analysis

Dry matter (DM) (AOAC 930.15) and mineral contents were determined in all diets and excreta. The samples were wet acid digested with nitric and perchloric acid mixture, and concentrations of P, K, Ca, Mg, Na and Fe were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument. The concentrations of Cu, Mn and Zn were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using a Perkin Elmer Elan 6000 instrument.

5.3.3 Calculations

The apparent mineral retention was calculated using the following formula, with appropriate corrections for differences in DM content.

$$\text{Retention coefficient} = \frac{[(\text{Feed intake x Mineral}_{diet}) - (\text{Excreta output x Mineral}_{excreta})]}{\text{Feed intake x Mineral}_{diet}}$$

5.3.4 Data Analysis

The data were subjected to repeated measures analysis using the General Linear Model of SAS® (SAS Institute, 1997) to assess cereal differences over time, and cereal x age interactions.

5.4 RESULTS

The determined mineral content of the diets is shown in Table 1.

Table 1: Determined mineral contents of diets based on wheat, sorghum and maize

Mineral	Wheat	Sorghum	Maize
Calcium (g/100g)	0.98	1.07	1.15
Phosphorus (g/100g)	0.78	0.72	0.84
Potassium (g/100g)	0.97	0.94	1.06
Sodium (g/100g)	0.20	0.18	0.21
Magnesium (g/100g)	0.20	0.19	0.20
Iron (mg/kg)	203	189	239
Manganese (mg/kg)	163	148	174
Zinc (mg/kg)	156	150	158
Copper (mg/kg)	24.5	28.2	21.3

5.4.1 Major minerals

The total tract mineral retention for major minerals in young broilers from days 3 to 14 post-hatch is shown in Table 2. The retention for individual minerals differed widely, ranging from 0.31 for potassium to 0.64 for sodium at day 14. Cereal effect was significant ($P < 0.05$) for calcium, phosphorus and sodium with sorghum diets generally having greater mineral retention than maize or wheat diets. Age effects were significant ($P < 0.05$) for all minerals, with mineral retention highest at day 3 for phosphorus, potassium and sodium, and highest at day 5 for calcium. In general mineral retention declined from day 3 to day 7 after which no changes were observed to day 14. Decline in mineral retention with age was similar across all three diets as shown by non-significant ($P > 0.05$) cereal x age interaction.

Table 2. Least square means for total tract retention of major minerals in broilers fed wheat, sorghum and maize-based diets during the first 14 days post-hatch

Mineral	Diet	Day 3	Day 5	Day 7	Day 9	Day 14	Pooled SEM
Calcium	Wheat	0.39^{a12}	0.47^{3}	0.35^{1}	0.41^{2}	0.44^{23}	
	Sorghum	0.51^{b12}	0.55^{2}	0.52^{12}	0.50^{12}	0.49^{1}	0.021
	Maize	0.43^{a}	0.45	0.40	0.42	0.40	
Phosphorus	Wheat	0.59^{a3}	0.54^{a3}	0.44^{a1}	0.47^{a12}	0.50^{2}	
	Sorghum	0.66^{b3}	0.60^{b2}	0.54^{b1}	0.52^{b1}	0.53^{1}	0.016
	Maize	0.60^{a3}	0.55^{a2}	0.47^{a1}	0.49^{ab1}	0.49^{1}	
Potassium	Wheat	0.47^{2}	0.35^{1}	0.30^{1}	0.32^{1}	0.32^{1}	
	Sorghum	0.47^{3}	0.37^{2}	0.30^{1}	0.34^{12}	0.30^{1}	0.021
	Maize	0.49^{3}	0.38^{2}	0.34^{12}	0.35^{12}	0.30^{11}	
Sodium	Wheat	0.92^{3}	0.58^{a12}	0.54^{a1}	0.56^{a12}	0.60^{a2}	
	Sorghum	0.93^{3}	0.67^{b2}	0.61^{b1}	0.62^{b1}	0.65^{b12}	0.017
	Maize	0.95	0.68^{b}	0.66^{b}	0.63^{b}	0.68^{b}	

^{a,b} Different superscript letters within a column are statistically different ($P < 0.05$).

^{1,2,3} Different superscript numbers within a row are statistically different ($P < 0.05$).

5.4.2 Minor minerals

The total tract retention of minor minerals in young broilers is shown in Table 3. Retention for minor minerals was lower than those determined for major minerals and the retention coefficients ranged from almost zero for zinc to 0.24 for magnesium at day 14. Cereal effect was significant (P < 0.05) for all minerals, with sorghum diets having greater mineral retention than maize or wheat diets, and a numerical tendency for maize diets to have higher mineral retention than wheat diets. Age effects were significant (P < 0.05) for all minerals, with mineral retention highest at day 3, declining to day 5 and then remaining unchanged to day 14. Cereal x age interaction was not significant (P > 0.05), indicating that the decline in mineral retention with age was similar across all three diets.

Table 3. Least square means for total tract retention of minor minerals in broilers fed wheat, sorghum and maize-based diets during the first 14 days post-hatch

Mineral	Diet	Day 3	Day 5	Day 7	Day 9	Day 14	Pooled SEM
Magnesium	Wheat	0.34^{2}	0.25^{a1}	0.21^{1}	0.24^{1}	0.24^{1}	
	Sorghum	0.39^{3}	0.33^{b23}	0.26^{1}	0.27^{12}	0.24^{1}	0.024
	Maize	0.39^{2}	0.29^{ab1}	0.26^{1}	0.27^{1}	0.23^{1}	
Iron	Wheat	0.26^{2}	0.20^{2}	0.11^{a1}	0.19^{ab12}	0.21^{b2}	
	Sorghum	0.27^{3}	0.19^{23}	0.11^{a12}	0.12^{a1}	0.06^{a1}	0.029
	Maize	0.34^{2}	0.20^{1}	0.21^{b1}	0.24^{b1}	0.21^{b1}	
Manganese	Wheat	0.17^{a2}	0.11^{a1}	0.02^{a1}	0.08^{12}	0.08^{12}	
	Sorghum	0.32^{b3}	0.24^{b23}	0.31^{b2}	0.11^{1}	0.13^{12}	0.046
	Maize	0.25^{ab2}	0.13^{ab12}	0.11^{a1}	0.17^{12}	0.11^{1}	
Zinc	Wheat	0.24^{a3}	0.10^{a2}	0.04^{a12}	0.08^{12}	-0.01^{1}	
	Sorghum	0.43^{b3}	0.25^{b2}	0.21^{b2}	0.11^{1}	0.05^{1}	0.036
	Maize	0.28^{a3}	0.13^{a2}	0.10^{a2}	0.13^{2}	0.00^{1}	
Copper	Wheat	0.31^{b3}	0.19^{a2}	0.18^{b2}	0.08^{1}	0.21^{b2}	
	Sorghum	0.50^{c3}	0.44^{b23}	0.40^{c2}	0.11^{1}	0.38^{c2}	0.029
	Maize	0.23^{a3}	0.12^{a12}	0.08^{a12}	0.13^{2}	0.04^{a1}	

^{a,b} Different superscripts letters within a column are statistically different (P<0.05).

^{1,2,3} Different superscript numbers within rows are statistically different (P<0.05).

5.5 DISCUSSION

Mineral retention coefficients determined in the present study were similar to those determined by Ravindran (2004) for 28-day old broiler chickens with the exception of calcium which was lower (0.45 versus 0.73). Ravindran *et al.* (2006) determined the apparent ileal digestibility of minerals for 21-day old broiler chickens. The major differences between apparent ileal digestibility coefficients and retention coefficients are method of determination (marker vs. total excreta collection) and site of determination (ileal vs. excreta). Data from this trial showed similar digestibility and retention coefficients for calcium, phosphorus iron and manganese, lower coefficients for potassium, and zinc and higher coefficients for magnesium, copper and sodium. Notably the retention coefficient was much higher (0.68) than the ileal digestibility coefficient (-0.30) for sodium, suggesting that significant re-absorption of sodium is taking place in the large intestine.

No published data are available on the pattern of mineral retention over the first 14 days of age in broiler chickens. Data presented in Chapter 4 of this thesis showed a decline in AME_n from day 3 to day 7 in a pattern similar to the decline in mineral retention measured in this study. However, AME_n data in chapter 4 showed a subsequent increase in AME_n to day 14, which was not observed for mineral retention in the current study. Furthermore, the higher mineral retention pattern observed in the sorghum diet was not replicated in AME_n values. It is notable that a number of studies have shown a decrease in nutrient digestion over the first several days post-hatch in a similar pattern to the mineral retention pattern measured in this trial (Murakami *et al.* 1992, Zelenka, 1968).

Mineral retention may be improved with the use of exogenous enzymes in broiler diets. The use of exogenous phytase is now a widespread commercial practice to improve phosphorus digestibility in broiler diets. Furthermore, Ravindran (2004) showed that the addition of phytase to broiler diets improved the digestibility of calcium, phosphorus, potassium, magnesium, iron and zinc.

Recently the use of chelated minerals has been shown to improve mineral retention (Paik *et al.*, 1999; Swiatkiewicz, 2001). This is due to organic minerals being more bioavailable than non-organic minerals. The use of organic minerals may provide greater mineral retention, allowing the requirements of the growing chicken to be met with lower overall mineral levels in diets.

Meeting the mineral requirements of the newly hatched chick is important in modern broiler management. Accurate mineral retention data over this period will help to define mineral requirements and assist in the formulation of appropriate diets for chicks over the first two weeks post-hatch.

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Chapter 6

Summary and Conclusions

6.1 SUMMARY

The choice of cereal base in pre-starter diets may be critical during feed formulation to maximise nutrient availability and bird performance. This study investigated cereal and age related differences in nutrient utilisation in the young broiler chicken, and the interaction between them. Wheat, sorghum and maize are the main cereals used in the New Zealand broiler industry. Diets based on these cereals were fed to broiler chicks and cereal effects on performance, nutrient utilisation, digestive tract development, and gut morphology assessed.

It is known that different grain types affect the growth rate and feed efficiency of broiler chickens (Ao and Choct, 2004). In the present study, chicks fed the maize based diet grew faster and had a numerically lower feed per gain than those fed the sorghum based diet. The performance of birds fed the wheat based diet was not significantly different from either the maize or sorghum based diets. Comparing the performance of chicks fed wheat, sorghum and maize based diets, Ao and Choct (2004) found that sorghum and maize fed birds grew faster and had better feed efficiency than those fed a wheat based diet, and both Ao and Choct (2004) and Tulasi *et al*., (2004) found that maize fed chickens grew more efficiently than sorghum fed chickens. The poor performance of the sorghum based diet in this study may have been due to the presence of tannins which can reduce protein digestibility and feed efficiency. The higher than expected performance of the wheat based diet may be due to the addition of exogenous xylanase in this study. In addition, all three grains are known to vary widely in AME due to a number of factors including season, variety and storage (Connor *et al.,* 1976;

Hughes and Choct, 1999). In summary, with the exception of the higher than expected performance of the wheat based diet, the results were as expected.

The gastrointestinal tract is a major consumer of nutrients in animals, with rapid cell turnover utilising significant quantities of both nutrients and energy and an increase in the proportional size of the gastrointestinal tract will therefore reduce feed efficiency. In this study the major organs of the digestive tract as measured on day 14 did not show any treatment differences. Villus height and crypt depth were also similar on day 14. High NSP cereals such as wheat may increase either the size of the gastrointestinal tract, or the rate of cell turnover in the gut (Jorgensen *et al.*, 1996; Iji, 1999), but this was not observed in the present study. This may have been due to a large variation between chicks within treatments, suggesting that a large sample size would be necessary before treatment differences could be detected. Furthermore, the addition of exogenous xylanase may have ameliorated any anti-nutritive effect of wheat NSP.

Viscous grains with relatively high levels of non-starch polysaccharides (NSP) such as wheat are known to have lower AME than non-viscous grains such as maize and sorghum which contain low levels of NSP (Choct and Annison, 1990), and the anti-nutritive effects of NSP may be greater in young birds. Both experiments in this research study showed significant differences in AME between cereals, with the wheat based diet tending to have a lower AME_n than those of maize and sorghum based diets. These results are consistent with expected results and suggest that the use of non-viscous cereals in pre-starter diets may be beneficial. The decline in AME observed between days 5–9 post hatching needs to be further investigated, as the first 10 days now comprise 30% of the total growth period of the modern broiler in New Zealand. Furthermore, the possibility of compensatory growth ameliorating the effects of early performance needs to be investigated.

In the present study, total tract digestibility of starch in all three diets was determined to reach values of 95% by day 5 post-hatching. Overall digestibility results are consistent with previous reports (Uni *et al.*, 1995; Noy and Sklan, 1997) with total tract starch digestibility of over 95% by 4-10 days post-hatching. However, digestibility declined from day 5 to day 7, before increasing again to day 14. Furthermore, the modifying effect of caecal microflora on nutrient digestion is now well documented (Ravindran *et al.*, 1999) and nutrients that disappear post-ileum are not considered beneficial to chickens (Ravindran and Bryden, 1999). The present study utilised total tract digestibility, and it is possible therefore that starch utilization has been overestimated and, in future studies, nutrient digestibility must be measured at the terminal ileum rather than in the excreta

Studies have shown that digestion of both unsaturated and saturated fats is low in young chicks (Carew *et al.*, 1972; Polin and Hussein, 1982; Sell *et al.*, 1986) and this poor digestibility has been attributed to low lipase activity and/or to insufficient bile secretion. The results from the present study also confirm that fat digestibility is low in the young chick. Total tract digestibility of fat reached a low point of 42% for the wheat based diet on day 7, before increasing to 76% by day 14, suggesting significant inefficiency in dietary fat utilization in the newly hatched chick. The fat digestibility estimates determined in the current study during week 1 post-hatching are considerably lower than the value of 86% determined for maize oil in 2 to 7 day-old chicks by Carew *et al.* (1972). Therefore the addition of lipase enzymes to diets for young chicks may be beneficial and needs to be further investigated.

Accurate data on mineral retention by the young chick are necessary if diets are to meet the requirements of the chick during the early stage of development, and at the same time minimise the environmental cost of waste disposal. Currently there is no published data on chick mineral retention over the first 14 days of age (Ahmad and Sarwar, 2006). Mineral retention coefficients determined in the present study showed a decline from day 3 to day 5 after which was no further change was detected.

6.2 CONCLUSIONS

The studies reported in this thesis investigated the performance and nutrient utilisation of broiler chicks fed diets based on wheat, sorghum or maize. Furthermore, it attempted to explain performance differences through a number of additional measurements. Performance and nutrient digestibility differences were seen between maize, sorghum and wheat based diets, but these differences were not related to digestive tract size or gut morphology. A significant reduction in nutrient utilisation over the period 5 – 9 days of age was observed suggesting that nutrient digestion and absorption is compromised during the first week of life of the broiler chick. A number of factors have been suggested as contributory factors, but further work is clearly required to identify the specific causes of the decline in digestibility during the early stages of chick development. In future evaluations it is important that the digestibility be estimated at the terminal ileal level rather than over the total tract. With the constantly reducing growing cycle of the modern broiler chicken, the early growth period is of increasing importance, and further work should focus around improving nutrient digestion over this period and in particular the drop in nutrient digestion observed between 5 – 9 days of age. Further research should include evaluating underlying biochemical and physiological events to identify those factors contributing to the cereal and age affects observed in this study. Obtaining maximum value from the relatively expensive New Zealand produced feeds through improved feed efficiency will improve the economic outcome for the New Zealand poultry industry.

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