CHAPTER 1 GENERAL INTRODUCTION

Grains and products of grains comprise 80 % or more of most diet formulations for poultry. Cereal grains are primarily used in animal diets as energy sources. To obtain the best value from grains, the characteristic of the grain should be matched with the digestive capacity of the bird. This can be done by grain selection and through further processing.

One third of the total cereal grains produced globally are used as animal feed of which around 60 % are used for poultry (Speedy, 2003). There are various cereal grain crops such as maize, wheat, rice, barley and sorghum grown all over the world. The contribution of maize in the animal industry is around 70 % (majority) as animal feed and the remainder is processed into a multitude of food and industrial products, including starch, sweeteners such as high fructose corn syrup, corn oil, and ethanol for use as a fuel (WIKI ANALYSIS, 2010).

Maize (*Zea mays*), the world's most widely grown (800 mt) cereal (FAO, 2007), is comparatively devoid of viscous non-starch polysaccharides (NSP), which are the main anti-nutritive factors present in most temperate cereal grains (Bedford, 1995; Smits and Annison, 1996; Bach Knudsen, 1997). However, in broiler chicken feeding trials, the terminal ileal digestibility of maize starch rarely exceeds 85 % (Noy and Sklan, 1994). The unaccessed starch at the later part of the ileum is supposed to be resistant to digestion and presents an opportunity to improve grain processing and inclusion of microbial enzyme supplements in maize-based diets. Among the cereals, maize is the most common energy feedstuff fed to poultry all over the world (Leeson and Summers, 1997), even though substantial amounts of wheat, barley, sorghum and rice/rice by-products are also used in poultry diets when price and supply allow for their inclusion. According to Metayer *et al.* (1993) maize is considerably less variable than other cereal grains in terms of protein content, ranging from 90 to 119 g/kg DM and consistently high in AME values, 15.6 ± 0.1 MJ/kg DM. However, its nutrient value can vary with season, variety and post-harvest processing (Iji *et al.*, 2003; Cowieson, 2005).

Cereals play an important role in feed formulation. It is essential to identify and evaluate less expensive, easily and locally available energy sources. The typical feed formulations for different categories of poultry are quite variable in their composition, depending on the availability of ingredients. A major problem faced by commercial nutritionists is the lack of specific information on the nutrient composition and anti-nutritive properties of feed ingredients that they may intend to use in the feed mill. It is assumed that an ingredient has the same properties consistently, regardless of the source, growing conditions and processing technique. Maize, for example, may be sun-dried or artificially dried by different processes, which would create variations in quality.

Both season and location can have major effects on starch, crude protein and AME contents of wheat, triticale and rye (Conan *et al.*, 1992; Metayer *et al.*, 1993). The effect of site of growth on starch and fibre contents of wheat grown in the UK has been demonstrated (Longstaff and McNab, 1986). A recent Australian survey of wheat over 3 years also revealed large variations in AME value, starch, protein, and soluble and insoluble NSP due to year of harvest and geographical locations of growth (Choct, 1999a). Other cereals such as barley, maize, oats and sorghum were relatively consistent across seasons and sites. However, the current literature indicates some variation/ inconsistency in nutrient composition for maize, sorghum and barley (Bryden *et al.*, 2009; Perez-Maldonado and Rodrigues, 2009).

Moreover, climatic conditions can affect starch content and granular structure, composition and distribution in the developing grain (Tester, 1997; Tester *et al.*, 1995), which could affect the nutritive value of cereal grains. Harvesting conditions such as stage of harvest and drying temperature can also affect starch content as well as composition in the grains.

The physical form of the grains used in poultry diets can have a bearing on the nutritive value, especially energy value. Particularly, fine grinding of grains is necessary to ensure thorough mixing with other ingredients. Kasim and Edwards (2000) reported that particle size of maize had a large effect on calcium and bone ash, phosphorus and phytate phosphorus vulnerability in broilers fed maize-soybean based diets low in phosphorus. Milling and grinding of grains serve the purpose of physical disruption to

the cellular structure of starch granules, thereby increasing the surface area for exposure to digestive enzymes. A combination of heat and moisture treatments by steam pelleting, expansion, or extrusion is used to gelatinize starch to improve its digestibility (Moran, 1982b). On the other hand, heat and moisture treatment can result in formation of resistant starch (Blakeney, 1993) and solubilisation of NSP (Vranjes *et al.*, 1994), which can have undesirable effects on animal nutrition. The benefits of physical processing of grains for monogastric diets are debatable and need further assessment.

In recent years, enzyme supplementation to reduce anti-nutritional effects such as those of NSP has been an active subject of research (Bedford and Morgan, 1996) and is now standard industrial practice in many areas when cereals are used. Cowieson *et al.* (2005a) observed that pelleting wheat-based diets above 80 °C can compromise bird performance and that if higher temperatures are to be employed, the use of exogenous xylanase is critical to maintain productivity. The digestibility of nutrients by broilers fed on maize-based diets can be improved by the use of a combination of xylanase, amylase, protease and phytase (Cowieson *et al.*, 2006a).

There is a dearth of research on the effects of pre- and post-harvest processing on maize quality, including the performance of broiler chickens fed on maize-based diets. The research by Iji *et al*. (2003) evaluated the effects of heat-treatment on maize grain that had been obtained from the same source and was uniform in quality. It is likely that maize from different growing environments would differ and these differences will increase with pre-feeding processing such as drying/processing temperature and milling technique.

The broad aim of this doctoral thesis is to compare the nutritive value of maize from various sources and optimize drying temperature and particle size to maximize the digestibility of the grain with the addition of microbial enzyme. The specific objectives of this research are:

1. To investigate the variations in nutrient composition of sun-dried maize grain from different locations when subjected to heating;

2. To examine the ultra-structural variation and nutrient composition of high-moisture maize grain when artificially dried at different temperatures;

3. To investigate the effect of milling technique and particle size on the nutritive value of maize grain from different sources;

4. To examine the response of maize inclusion level in diets with or without microbial enzymes, and

5. To investigate the effect of microbial enzyme supplements on diets based on maize when dried at different temperatures.

The feeding trials described in this thesis were conducted on broiler chickens from hatch to 21 days of age. Trials were not extended into the finisher phase, in order to exclude confounding effects of change in diets between feeding phases.

CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

Cereals are the most important crops in the world, with total annual grain yields exceeding 2700 million tonnes (mt) compared with less than 350 mt for legume seeds (FAO, 2007). A number of cereal species are grown, such as maize (800 mt) with the highest production in USA (Figure 2.1), wheat (700 mt) and rice (678 mt) which, in 2008-09, together accounted for over 80 % of the total production. Other cereals include barley (133 mt), sorghum (63 mt), and millet (*Panicum milaiceum)* (341 mt), while the production of oats and rye (*Secale cereal)* has considerably gone down (FAOSTAT., 2010). However, animals consume one-third of the global cereal grain supply. In a world where the human population is forecast to reach 7.7 billion by the year 2020, a fixed or possibly shrinking quantity of arable land, and an estimated 800 million undernourished people, quantifying the net contribution of animal production to quantity and quality of the food supply is important.

The most expensive component of feedstuffs for poultry, in terms of total amount in feed, is energy and large amounts of cereal grains are used for this purpose throughout the world. Australia alone uses about 12 million tonnes of cereal grains for livestock feeding each year, 19 % of which is used in the chicken meat industry.

Over the period of 15 years, calorie conversions have improved by 12 % from 6648 calories of feed per live kg to 5885 calories of feed per live kg worldwide. Every one percent of the improvement decreases calorie demand per kg of live weight by 60 calories. At current feed costs of $260 per ton or approximately $0.57 per live kg, a two percent improvement in feed cost per live kg in the US industry producing 22 billion kg is worth $127.4 million. The 12 % gain seen over the last 15 years at current feed costs has brought over $1.5 billion in benefits to the US industry alone. Worldwide, the effect would more than quadruple (FAO, 2007).

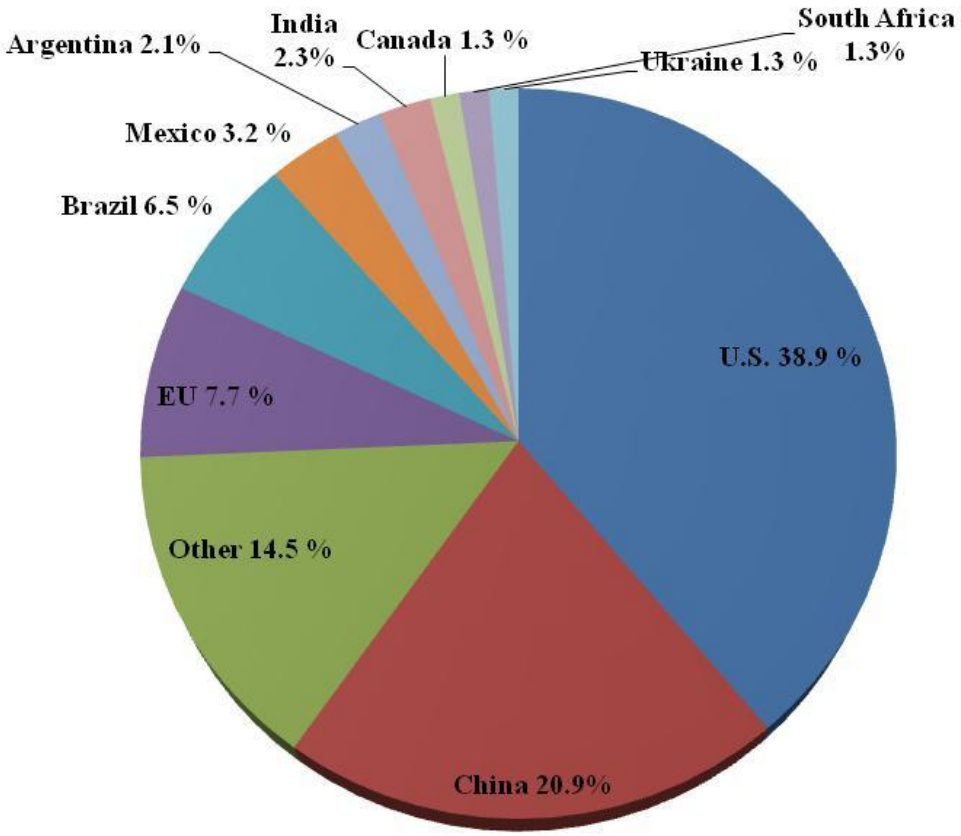


Figure 2.1 World maize grain production in 2008-2009 (% of a total production of 791 million tonnes), Source: USDA/Foreign Agriculture Service, Grain: World Markets and Trade, January 2009

As a result, it is not surprising that the efficiency of energy utilization is of vital importance in poultry nutrition and is usually measured by the amount of energy from the feed metabolized, which is referred to as apparent metabolizable energy (AME). The AME values of cereal grains, however, vary greatly, especially at the time of harvest. Thus, the value of newly harvested wheat/maize/sorghum as the main energy sources in poultry diets is not always similar to that of previously harvested or even stored grains (Reed, 1992; Hughes and Cooper, 2002). Another factor is that the nutritive value of cereal grains for poultry is inversely related to the level of NSP (Annison, 1991). The consequence of this is the widespread use of feed enzyme supplements to enhance nutrient digestibility in non-ruminant animals.

According to the FAO, the worldwide total production and use of animal feed exceeded 4000 mt in 2001, of which some 550 mt were milled feeds (Bruinsma *et al.*, 2002). There is a continuing rise in the demand for animal products and particularly those from poultry and pigs. The FAO and other institutions have suggested that global production of animal products will rise impressively over the next 20 years and animal feed ingredients will be in short supply especially cereal grain. It is well known that about two-thirds of global coarse grain production is used as animal feed with increasing price per unit. For example, the feed cost for the broiler industry alone is estimated to be $450–500 million per annum in Australia. With the above circumstances in mind, it is necessary to find a way to use cereal grains in poultry diet more efficiently, thus optimizing the supply in relation to demand. For this review of literature the focus will primarily be on the major cereal grains, and variation in chemical composition especially, carbohydrate and protein, and nutritive value.

2.2 CEREALS AS SOURCES OF ENERGY

The principal cereal crops grown in the world are maize, wheat, barley, rice, oats, rye, triticale and sorghum. These grains are nutritionally dense and supply carbohydrates and proteins as well as a variety of micronutrients, in particular certain B vitamins, vitamin E and minerals. Cereals are primarily used in animal diets as energy sources and the energy component represents the greatest proportion (around 70 to 75 %) of the dietary energy for poultry meat and egg production. Therefore, it is important to select the grain prudently for energy supply. Among the cereals, maize is the most common energy feedstuff fed to poultry worldwide (Leeson and Summers, 1997), although substantial amounts of sorghum, wheat, barley and rice/rice by-products are also used in poultry diets when price and supply allow for their inclusion. Other cereals fed on a much more limited scale include oats, rye, triticale and millet.

Effective feed evaluation systems are required in order to predict the performance of farm animals on different diets. The metabolizable energy is the system used predominantly as the feed evaluation parameter in most countries and is thus the basis of most feeding systems in poultry. It is expressed as apparent metabolizable energy

(AME) or true metabolizable energy (TME). Some argue that AME and TME should be corrected for zero nitrogen balance, thus the terms AMEn and TMEn.

2.2.1 Physical properties of cereal grains

The commercial value of grain is based on its physical quality, which determines the end use of the product. In terms of nutrient quality, the following physical properties are very important for maize grains, colour, size–shape (Figure 2.2), breakage, stress cracking, hardness, fungal contamination and seed viability. The colour of grain is an important quality factor that greatly influences industry demand as well as consumer acceptance (Mendoza *et al.*, 2006). The feed industry wants clean, brightly coloured maize for food products and the colour of maize kernels helps to determine the class of maize such as yellow, white, orange, red, purple and brown (Watson, 1987). Bright, clean, yellow and white (sometimes) kernels are preferred by the poultry industry.

Seed maize is also marketed by kernel size, and this is a very important characteristic for the seed maize industry but not as much in the poultry industry yet. An ear of maize contains a large number of kernels, each with a slightly varying physical size and shape. This reflects its position on the ear; seeds on the tip of an ear tend to be small and round, seeds in the middle of an ear tend to be flat and seeds on the bottom of an ear tend to be large and triangular. Maize kernels are classified as convex or dent, based on their crown-end shape. Recent practices of harvesting high-moisture maize introduce substantial mechanical damage to kernels, which is further aggravated by subsequent handling and transportation operations. It is estimated that on-farm mechanical damage (during harvesting) to maize kernel ranges from 20 to 80 % (Pierce *et al.*, 1991). Such damage includes kernels that have hairline cracks, and those that are broken, chipped or crushed. It has been reported that kernel breakage is greater at high plant densities (Bauer and Carter, 1986; Moes and Vyn, 1988; Vyn and Moes, 1988).

Internal damage in maize appears in the form of stress cracks in the endosperm (Thomson and Foster, 1963). Stress cracks originate in the inner core of the floury endosperm, and propagate rapidly outwards along the boundary of starch granules, but many of them do not advance as far as the pericarp layer.

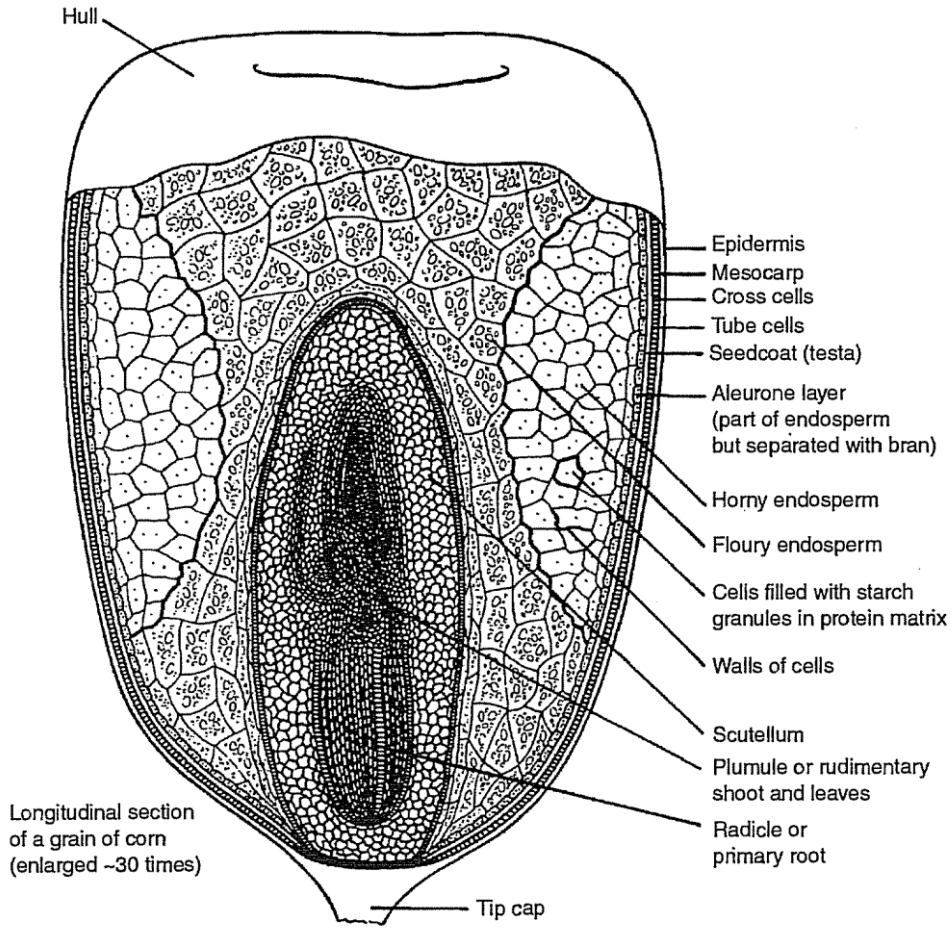


Figure 2.2 The structure of the maize grain– longitudinal section (Pomeranz, 1978).

Grundas and Pecen (2004) described some characteristics of the physical properties of a single kernel in the case of wheat heads. Commonly, internal cracks originate even in the pre-harvest period and lead to a crop reduction as a result of the weakening of the structure of the endosperm. Grain becomes softer and less resistant to dynamic loads inflicted by the active elements of harvesting and static or quasi-static loads during transport or storage. Fragmented grain is more susceptible to attacks by pests in storage bins. For future use, it is necessary to keep maize grain containing acceptable moisture content in storage for a long time. However, maize quality can be affected by mould and fungal contamination. Even under natural drying conditions in the field, protozoa have a chance to contaminate the grain, particularly in wet climates. Hardness or vitreousness is an important grain quality factor for maize, affecting milling characteristics and, conversely, grain particle size is dependent on milling. So, all of the above physical

properties of grains, in particular, maize are very important in terms of quality and nutritional value.

2.2.2 Nutrient/Chemical composition of cereals

2.2.2.1 Carbohydrate content

In general, carbohydrates constitute about 75 % of the solid content of cereals. In cereals, as in other plant tissues, carbohydrates are localized in (1) the cell wall, (there are especially thickened walls in supporting tissues of husk and seed coat) (2) plastids, where starch constitutes the largest proportion of carbohydrates in all cereals, and (3) in vacuoles or the cytoplasm.

The principal constituents of cell walls are cellulose, hemicelluloses, pectins, and lignin. The hemicelluloses are a heterogeneous group of polysaccharides that contain numerous kinds of hexose and pentose sugars and in some cases residues of uronic acids. These polymers are classified according to the predominant sugar residue and are individually referred to as xylans, arabinogalactans, etc. Cell walls are the main components of "dietary fibre". The biological availability of protein, minerals and other nutrients, such as vitamin B_1 in rice, may be reduced by its fibre constituents (Torre and Rodriquez, 1991). The principal carbohydrate of all cereals is starch, which is similar in composition, having 74–79 % amylopectin and 25–30 % amylose. High-amylose and high-amylopectin cereal cultivars have also been developed. The presence of lipid in cereal starches is a distinguishing feature of these starches (Morrison *et al.*, 1984). There is also considerable variation in the transition temperatures of starches within species. Interactions of cereal starches with protein and lipids are known to influence physicochemical characteristics such as gelatinization and retrogradation. Starch gelatinization is the disruption of molecular orderliness of starch granules. It results in granular swelling, crystallite melting, loss of birefringence, development of viscosity and solubilisation (Liu *et al.,* 2009). The variations in chemical composition of different cereal grains from different countries are summarized in Table 2.1, determined

Table 2.1 The chemical and amino acid composition (g/kg DM) of major cereal grains

Components	Maize		Wheat		Sorghum		Barley		Triticale	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
DM	881.0	905.0	884.0	911.0	870.0	892.0	880.0	896.0	905.0	912.0
Crude protein	71.3	137.0	87.0	209.0	78.4	133.0	94.9	115.0	112.7	117.3
Lipid	20.0	58.0	14.6	25.0	33.0	35.0	27.0	64.0	22.0	23.0
Crude fibre	16.0	19.0	27.0	28.0	17.0	23.0	29.0	53.7	40.0	45.0
Ash	11.0	16.0	11.0	19.0	10.0	12.0	18.5	23.8	18.0	19.0
Ca	7.0	7.5	3.4	5.0	2.8	2.9	0.1	0.2	0.4	0.5
P	1.3	2.8	2.2	3.9	1.9	4.1	2.0	2.9	1.2	1.8
NSP	85.0	95.0	90.0	128.0	40.0	50.0	167.0	411.0	95.0	163.0
Starch	640.0	800.0	650.0	732.0	532.0	700.0	513.0	642.0	563.0	629.0
ME (MJ/kg)	14.0	19.5	12.1	13.2	13.1	16.1	10.7	12.5	12.1	13.5
AME (MJ/kg DM)	12.2	17.0	10.35	15.9	13.5	17.7	10.4	12.2	8.6	16.2
Amino acids										
Arginine	4.1	4.7	7.3	9.2	3.6	5.2	4.1	5.5	5.0	6.0
Leucine	10.9	12.97	9.8	11.7	10.4	16.7	5.2	7.7	5.5	7.7
Lysine	2.2	3.2	4.4	5.1	2.4	3.7	3.4	4.0	3.2	4.1
Methionine	1.3	1.9	2.6	4.2	1.0	2.0	1.1	1.5	1.0	1.7
Phenylalanine	4.4	5.1	6.0	7.3	2.8	5.8	4.2	5.4	4.3	5.4
Histidine	2.4	3.2	3.0	3.5	1.2	2.1	2.1	2.6	2.2	2.9
Threonine	3.1	5.5	4.0	5.1	3.2	4.9	3.2	3.5	3.2	3.9
Tryptophan	0.6	0.7	1.6	2.4	1.0	1.6	1.0	1.2	0.89	1.1
Tyrosine	2.6	3.6	4.1	4.9	2.0	4.3	2.5	3.0	2.1	3.0
Valine	4.0	5.1	6.1	7.4	4.0	5.8	4.5	5.4	4.5	5.4

Sources: Guirguis, 1975; Connor *et al.*, 1976; Shingari *et al.*, 1976; Farrell, 1983; Mollah *et al.*, 1983; Rogel *et al.*, 1987; Chavan and Kadam, 1989; Englyst, 1989; Fox *et al.*, 1992; Eliasson and Larsson, 1993; Leeson *et al.*, 1993; Metayer *et al.*, 1993; Flores *et al.*, 1994; Hughes and Choct, 1997; Kocher *et al.*, 1997; Leeson and Summers, 1997; Chung and Ohm, 2000; Kasim and Edwards Jr, 2000; Brand *et al.*, 2003; Pirgozliev *et al.*, 2003; Holtekjolen *et al.*, 2006; Bryden *et al.*, 2009; Perez-Maldonado and Rodrigues, 2009.

by various researchers. It is clear that the chemical composition of cereals grains is highly variable.

Maize (*Zea mays*)

Maize is probably the most important cereal grain used for poultry feeding around the world and it is the principal grain in terms of volume (800 mt) of production (FAO, 2008). The gross AME value of maize is higher than that of barley and wheat because of its higher starch level (64–80 %) and lower NSP content (9.5 %) (Eliasson and Larsson, 1993) (Table 2.1). The main fibre components in maize are arabinoxylans but, unlike wheat, maize contains very little soluble NSPs (Choct, 2006).

The chemical composition of maize is favourable for inclusion in poultry diet. However, its nutritional value can vary substantially from batch to batch (D'Alfonso, 2002). Ileal digestible energy can also vary by 2.04 MJ/kg DM. In addition, its nutrient composition can vary due to growing condition, season, and variety. In Australia, Connor *et al*. (1976) observed AMEn values for maize (15.5 to 17.0 MJ/kg DM; mean value 15.7, n = 48). An analysis of USA maize samples from the same harvest year showed that ME values ranged from 12.2 to 14.5 MJ/kg (Leeson *et al.*, 1993). Conversely, Metayer *et al.* (1993) reported consistent AME values for maize (15.6 ± 0.1 MJ/kg DM, n = 40) in surveys of French cereals. Analysis of 220 samples showed that despite limited variation in starch content (2 %), the variation in the *in vitro* starch digestibility of maize was higher (16 %). This variation cannot be attributed to viscosity; although Cowieson (2005) confirmed that this could be due to inconsistent starch digestibility.

Wheat *(Triticum aestivum)*

In terms of world production, wheat ranks as number two among the feed grains, with 700 million tonnage in 2007 (FAO, 2007). It is the most common grain in Australia and Europe. Wheat is an excellent source of energy. However, its nutrient composition can also vary due to variety. For example, the AME of Australian wheats for meat chickens varies widely (10.35 – 15.9 MJ/kg DM) (Mollah *et al.*, 1983; Rogel *et al.*, 1987) (Table 2.1). More recent studies with broilers (Choct, 1995; Hughes *et al.*, 1996; Hughes and Choct, 1997) have also confirmed that the AME of Australian wheat varies widely (10 –

15 MJ/kg DM). In contrast, are the consistently high AME values (14.5 ± 0.2 MJ/kg DM, n = 70) observed by Metayer *et al*., (1993) in annual surveys in France.

Overall, it can be summarized that the AME values of wheat for broiler chickens are variable, particularly for Australian wheats.

Barley (*Hordeum vulgare*)

Barley ranks number three among the feed grains after wheat and maize, with tonnage of 133 mt in 2007. It is the most common feed grain in Europe, where it is grown on relatively poorer soil than that used for other grains. Barley is considered a low energy feed grain due to its high fibre content as well as low metabolizable energy (12.5 MJ/kg) content. Recent analysis of a large number of barley samples by Holtekjolen *et al.* (2006) showed that starch is the major constituent of barley (51.3 – 64.2 %), followed by NSPs (22.6 – 41.1 %) (Table 2.1). The major fibre components of barley are β-glucans and arabinoxylans, which are mainly located in the endosperm cell walls and aleurone layers. The concentration of soluble NSPs in barley is two to five times higher than in wheat (Choct, 2006; Holtekjolen *et al.*, 2006).

Results from a broiler study (Kocher *et al.*, 1997) indicate that the AME of Australian barley also varies widely (10.4 – 12.2 MJ/kg DM), even in the presence of commercial β-glucanase (13.2 – 15.5 MJ/kg DM). Metayer *et al.* (1993) reported higher AME values in French barley (13.5 ± 0.2 (n = 107) and 13.1 ± 0.3 MJ/kg DM (n = 36) for 2-row and 6-row varieties, respectively) than those observed in Australia. The large between-bird variation seen for wheat-based diets is also apparent in barley diets. Hughes *et al.* (unpublished data) demonstrated that, when a barley-based diet was fed to 96 individually housed broilers (24 – 31 days of age) under identical conditions, the AME value ranged from 12.24 to 13.54 MJ/kg DM, exhibiting a 1.3 MJ difference due purely to individual bird variation.

Sorghum (*Sorghum bicolor*)

In many parts of the world, low tannin sorghums have similar nutritional value to maize and offer an excellent alternative ingredient for preparing diets for the production of non-pigmented poultry products (Nyachoti *et al.*, 1997; Leeson and Summers, 2001). In

many areas of Australia, sorghum is preferred to wheat for poultry feeding due to its consistent AME and lower price. The recent work of Perez-Maldonado and Rodrigues (2009) on the evaluation of Australian sorghum showed a starch mean value of 60 – 68 % DM and 53 – 70 % for 2004 and 2005, respectively (Table 2.1). Since starch is considered to contribute about 70 % of the total energy of the grain, variation in grain starch content may be reflected in the AME values. It is also reported that sorghum contains resistant starch, which can impair its digestibility and which has been associated with tannin and an excessively hard peripheral endosperm layer (Rooney and Pflugfelder, 1986). Metayer *et al.* (1993) reported consistently high AME values for sorghum (16.1 ± 0.1 MJ/kg DM, n = 10) in surveys of French cereals. In Australia, Connor *et al.* (1976) observed AMEn values for sorghum ranging from 13.5 to 17.7 MJ/kg DM (mean value 15.8, n = 39). Hughes and Zviedrans (1999; unpublished data) observed AME values ranging from 14.9 to 15.8 MJ/kg DM in 12 samples of sorghum and 14.9 to 15.4 MJ/kg DM for ileal digestible energy (DE) for the same sorghum samples. So the ME value of sorghum can vary due to its source and location.

Triticale (*X-Triticosecale*)/ (*Triticale hexaploide Lart.*)

Triticale is also an important feed grain with a similar nutrient composition to wheat. Shingari *et al.* (1976) reported AME of triticale ranging from 8.6 to 14.1 MJ/kg in hens and meat chickens, although it is unclear from their paper whether similar degrees of variation in AME were evident in both types of poultry. Metayer *et al.* (1993) observed a range of 13.9 – 14.6 MJ/kg DM in 130 samples grown in France. Flores *et al.* (1994) reported on the wide variation in starch (56.3 – 62.9 % DM) and water-soluble pentosan content (2.77 – 5.09 % DM) in 18 triticale varieties grown at Cordoba in Spain (Table 2.1). Starch digestibility was high (96.5 – 99.4 %). The TMEn ranged from 14.0 to 15.2 MJ/kg DM in cockerels and from 13.6 to 14.9 MJ/kg DM in broiler chickens. The differences between young and adult birds in TMEn and starch digestibility were not consistent for all varieties. The AME of 47 Australian triticale samples ranged from 13.6 to 16.2 MJ/kg DM in adult cockerels (Farrell, 1983). Johnson and Eason (1988) reported a narrower range (12.8 – 14.3 MJ/kg DM) in eight samples grown on the same site and they attributed this to poor growing conditions as indicated by low grain densities (66.1 – 74.8 kg/h L air dry basis).

Oats (*Avena sativa*)

About 95 % of the world's oat crop is used for livestock feeding (McGee, 1984). However, oat consumption as human food has recently increased to 19 % in the United States (Bowers, 1992), perhaps due to the reported health benefits of the soluble fibre of oats. Oats thrive in a moist, cool climate and became an important crop in Northern Europe at the beginning of the seventeenth century. Oats have a relatively minor status among cereals because they are more difficult to process and are unstable due to their high lipid content and lipase activity. Metayer *et al.* (1993) observed a ME range of 10.5 – 11.4 MJ/kg DM in eight samples of black oats, and 11.6 – 12.4 MJ/kg in eight samples of white oats grown in France. Australian oats have not been studied as systematically. AME values for three Australian oat varieties ranged from 11.8 to 12.4 MJ/kg (Hughes *et al*., 2001).

The starch content of the various grains is best summarized by Choct and Hughes (2000) who reported that sorghum starch content was higher than that observed in wheat (65 %), rye (60 %), and barley (55 %) but lower than that of maize (75 %) and rice (80 %).

2.2.2.2 Protein content

The protein content of poultry feed is a major consideration in its cost and in its nutritional value to the bird. In its simplest form, this is expressed as the percentage of crude protein (CP) in the diet. However, Classen and Stevens (1995) opined that chickens use the digestible amino acids, which make up CP and as the nutrient requirements for poultry became ever more precise, protein availability and amino acid balance in a diet have become even more important. Soyabean meal, meat meal, fish meal and, increasingly, canola meal are used as protein sources in poultry diets. The cereals making up the majority of the diet are primarily considered to be energy sources. However, they can also contribute a substantial percentage of the desired protein (McNab, 1991). The CP content is also variable due to its source, variety and processing condition (Table 2.1). In a comprehensive survey of French cereals, Metayer *et al.* (1993) observed a wide variation in protein content (11.7 to 15.3 % DM) over seven seasons. They attributed the variation to a combination of genetic effects, climate, and

use of nitrogen fertiliser. Within a season, standard deviations were about ± 0.8 % protein, which means that the variations are attributed mostly to seasons.

Maize has considerably less variation, with protein content ranging from 8.8 to 12.2 % DM, irrespective of growing region, compared with wheat and barley. Similarly, Metayer *et al.* (1993) observed ranges of 9.0 to 11.9 % DM in protein content for maize grown in France over two to five seasons. However, recent data showed comparatively less variation in CP content (8.5 to 9.8 %) in maize from Australia (Bryden *et al.*, 2009) than the French findings. However, Metayer *et al.* (1993) observed slightly higher CP content in sorghum grown in France than in maize, with a range from 7.0 to 13.7 %.

The protein content can vary greatly between wheat cultivars, and between the regions in which they are grown. Mollah *et al.* (1983) confirmed the CP levels of 22 wheat samples between 11.4 to 18.0 %. The capability of broiler chickens to extract and use the CP and amino acids contained in feed ingredients is also variable. There are reports that the protein content of cereals is more source-dependent than the starch content and this difference depends on the supply of starch and protein precursors (Jenner *et al.*, 1991).

Boila *et al.* (1996) examined the variability in protein and amino acid contents of Canadian wheat (3 cultivars) and barley (3 cultivars) grown at 12 locations in Manitoba over three consecutive years. The effect of cultivar on protein content was significant for wheat but not for barley. Significant differences between cultivars were detected for several amino acids but were not regarded as critical for meeting requirements for essential amino acids in poultry and swine. Positive correlations between protein and individual amino acid content varied widely (R^2 = 0.29 to 0.88) probably due to the combined effects of location and year of growth. Interactions between location and year had much greater effect on individual amino acids than on protein. The proportion of lysine in total protein decreases as grain protein content increases (Boila *et al.*, 1996). In Australia, variation in protein content is as wide as that observed in European and North American studies (Hughes *et al.*, 1996; Choct *et al.*, 1999a). In the study by Choct *et al.*, (1999a), protein content ranged from 11.1 to 20.5 % DM in a total of only 15 general-purpose and feed grade wheats grown in the five mainland states of Australia in the same season. It could be anticipated that similar patterns of variation exist in other

cereal grains if these are also grown in a wide range of climatic conditions around the world, including Australia.

2.2.2.3 Lipid content

Lipids are important nutritional components in feed grains. In cereal grains, lipids are located mostly in the membranes and spherosomes. Lipids yield 2.25 times the energy per unit weight of carbohydrates and protein, provide essential fatty acids, add flavour factors, improve the efficiency of feed conversion and reduce the dustiness of the milled grains. Total lipid content of the cereal grains are oats, 5 – 9 %, maize kernels, 3.9 – 5.8 %, barley, 3.3 – 6.4 %, rye, 2.0 – 3.5 %, millet, 4 – 5.5 %, wheat, 2.3 – 2.5 % and rice, 0.8 – 3.1 % (Morrison, 1978; Youngs, 1986; Chung and Ohm, 2000) (Table 2.1). Lipid composition varies considerably within a cereal grain. Maize and sorghum have a high neutral lipid and low glycolipid content. The small grain varieties have a more balanced distribution of neutral lipids, glycolipids and phospholipids. However, the fatty acid composition of the total lipid is more or less similar for all grains (Price and Parsons, 1975).

The polar lipid content of oats is greater than that of other cereals since much of the lipid fraction is contained within the endosperm. In most cereals, the lipid fraction is concentrated in the germ and in the bran-milling fractions. About one-third of oat lipids are polar (8 – 17 % glycolipids and 10 – 20 % phospholipid). On the other hand, maize lipids are predominantly acyltriglycerides in cultivars having high total lipid content. The distribution of lipid classes is similar in wheat, barley and rye, which contain about 65 – 78 % nonpolar lipid, 7 – 13 % galactolipid and 15 – 26 % phospholipids (Morrison *et al.*, 1984). In wheat, the glycolipids play an important role in gluten development during bread-making (Pomeranz and Chung, 1978). The major fatty acids (Table 2.2) in cereal grain lipids are linoleic, oleic and palmitic (Haard and Chism, 1996).

Table 2.2 Principal fatty acids (%) of some cereal oils (Haard and Chism, 1996)

Fatty acid	Maize	Wheat	Rye	Rice
C:14:0	-	3	6	1
C:16:0	6	18	11	28
C:18:0	2	7	4	2
C:18:1	44	31	18	35
C:18:2	48	57	35	39
C:18:3	-	4	7	3

2.2.2.4 Mineral content

Minerals are needed for all normal life processes and the physiological importance of minerals for farm animals, including poultry, is well documented (Spears, 1999; Underwood and Suttle, 1999). Poultry derive the minerals required for normal growth and metabolism from the diet. The biological availability of a mineral from the diet is manifested by the efficiency with which the body utilizes and retains the dietary mineral. The retention will be influenced by a number of dietary factors, including diet or ingredient type, source of minerals and, levels and relative proportions of various minerals. The major essential minerals are Ca, P, Mn, Na, K and Cl and cereal grains contain these in varying proportions, according to type of cereal grain (Table 2.3).

Generally calcium and phosphorus constitute over 70 % of body ash and they are closely associated with each other in their metabolism and utilization especially in bone formation (Singh and Panda, 1992). The phosphorus in poultry diet is, in general derived from feedstuffs of both plant and animal origin and from supplements of inorganic phosphate. Plant phosphorus occurs mostly as phytin, the calcium, magnesium and potassium salt of inositol hexaphosphoric acid, which is hydrolyzed by phytase to yield inositol and inorganic phosphate (Karunajeewa, 1976). Although P is very critical for poultry, approximately two thirds of the P in cereal grains and oilseed meals is present in the form of P bound to phytic acid (Phytate P), which is not digested by poultry but a large proportion is excreted via the faeces.

Table 2.3 Key mineral composition of cereal grain dry matter (Singh and Panda, 1992)

Minerals	Maize	Wheat	Sorghum	Barley	Oats
Calcium (g/kg)	2.5	1.8	1.8	2.9	1.1
Phosphorus (g/kg)	4.0	4.3	3.2	6.3	4.1
Copper (mg/kg)	4.0	10.8	15.8	8.5	6.6
Iron (mg/kg)	30	56.0	-	60	79.0
Manganese (mg/kg)	4.8	57.0	16.3	18.3	42.9
Zinc (mg/kg)	12.1	15.6	15.4	17.2	-
Phytic acid (mg/100g)[1]	698±6.0	889±4.2	912±5.2	-	-

[1] Jood *et al.* (1995)

Phytic acid or phytate is a naturally occurring organic complex found in plants (Figure 2.3). Also known as inositol hexaphosphate (IP6), it is the principal storage form of phosphorus in many plant tissues, especially in the grass family (maize, wheat, rice, rye, barley, etc) and beans. Phosphorus in this form is generally not bioavailable to non-ruminant animals and humans because animals lack the digestive enzyme, phytase, required to separate phosphorus from the phytate molecule.

Phytic acid binds to important minerals such as calcium, magnesium, iron and zinc and can therefore contribute to mineral deficiencies, as the minerals are not released from the phytate and are thus unavailable to the body. As a reactive anion, it forms a wide variety of insoluble salts with divalent and trivalent cations. Phytic acid is also known to complex with proteins and consequently reduces their availability. In developing countries, a common way to increase the bioavailability of minerals from grains and beans is to ferment the material. Many bacteria possess phytase activity and by fermenting grains or beans by lactic acid bacteria the phytate is destroyed and the bioavailability of the minerals is increased.

Recent studies indicate that phytic acid reduces the activity of pepsin, trypsin and α-amylase. The detrimental effects of phytic acid in plants on poultry nutrition and the ameliorating effects of microbial phytase supplementation were reviewed by Sebastiana *et al.* (1998). The two main factors highlighted in this review were, firstly, the effects of phytic acid complexes on bioavailability of essential nutrients, and secondly, the likelihood that phytic acid has a sex-dependent effect on nutrient availability, with males showing reduced responses to dietary phytase supplementation.

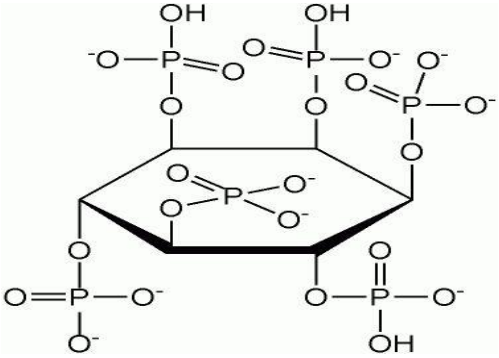


Figure 2.3 Structure of phytic acid (IP6)

Phytate P digestion by broiler chickens fed on commercial diets was much lower than digestion by chickens fed on a corn-soybean meal-based P-deficient diet (Edwards *et al.*, 1999). Use of microbial phytase is very popular in Europe and North America for reducing the need for expensive dietary phosphorus supplements, and for reducing the problem of waste disposal from livestock (Khan, 1996). Australian chicken growers are likely to follow this trend, probably more for the purposes of reducing feed costs and for improving the digestibility of grains with high phytic acid content than for waste disposal, whereas this latter benefit is more likely to be the main point of interest to egg producers in the immediate future. Published data on the effects of diet type, however, are limited. To our knowledge, no previous study has compared the mineral retention by poultry from diets based on the three most commonly used cereal grains, namely, wheat, sorghum and maize. Despite the biological and economic importance, published data on the utilization and retention of minerals in modern strains of poultry are limited.

2.3 FACTORS AFFECTING CEREAL QUALITY

2.3.1 Grain type and variety

It has been reported that the chemical composition and nutritional quality of maize, wheat and barley varies between varieties or hybrids (Bullock *et al.*, 1989) and the source of grain (Fuller *et al.*, 1989). The physical quality of maize also varies between maize hybrids due to variation in the proportion of kernels exhibiting stress cracks and kernel breakage (Vyn and Moes, 1988). Differences in grain quality characteristics between old and more recent varieties or hybrids could also be associated with

differences in stress tolerance and may vary with the plant density and other conditions under which the maize is grown. However, the changes in grain chemical and physical quality on different ears of maize hybrids have not been closely examined.

The protein in grains is low in lysine, an essential amino acid. The amount of lysine present varies between different grains and between different components of the grain. The content of lysine in the aleurone and germ is greater than in the endosperm. Maize is also low in the amino acid tryptophan. Neryng and Reilly (1984) stated that the variety of the maize selected has perhaps the strongest influence of all on yields, purity and ease of milling. Sorghum hybrids differed in AME, whereas there were no differences between maize hybrids. Moreover, differences between sorghum hybrids were not consistent over different growth sites, nor over seasons. Nyirenda *et al.*(1987) provided supporting evidence of seasonal and varietal effects on crude protein, ash, starch, crude fat, free sugars, tannins and AME of sorghum.

Johnson and Eason (1988) found significant differences in AME and digestibilities of dry matter, nitrogen and amino acids among eight different Australian varieties of triticale grown under relatively poor conditions on the same site in 1982. Similarly, Hughes (unpublished data) observed significant differences in AME (10.1 and 12.5 MJ/kg DM, measured within 6 weeks post-harvest) between Janz and Trident wheat varieties grown on one site during a season with hot, dry weather (1994 in South Australia). Under better growing seasons, differences between varieties are usually smaller. Indications are that in pinched wheat, the degree of grain-fill is partially responsible for differences in AME, which could be due to reduced starch content, or a higher proportion of cell wall content in the sample, or a combination of these two factors. Further studies are warranted in this area.

Wiseman (1993) found a significant interaction between variety and site among eight wheat varieties grown on two sites in the UK when fed to pigs and poultry. The effect of variety was evident on both pigs (15.0- 15.9 MJ/kg DM) and poultry (9.0 - 13.7 MJ/kg DM), with the latter showing a noticeably greater variation. It is worth mentioning that one variety of wheat had very low AME values (7.7 and 10.3 MJ/kg DM), indicative of extreme cases of low-ME wheats, but not remarked upon as such by Wiseman (1993).

The study by Boila *et al.* (1996) showed that Canadian barley cultivars were less variable in protein than wheat cultivars, but both grains exhibited wide variation in positive relationships between total protein content and amino acids due to location and year of growth. On the other hand, Metayer *et al.* (1993) observed mean protein values of 12.2 and 11.4 % DM with ranges of 10.0 to 15.6 % and 9.5 to 14.0 % for 2- and 6-row barley, respectively, in France. For triticale, Metayer *et al.* (1993) reported a mean protein value of 12.2 % DM (8.7 to 16.1 % DM) in 130 samples collected over four growing seasons in France. Flores *et al.* (1994) also reported wide variation (14.9 to 20.3 % DM) in the protein content of 18 triticale varieties grown in Spain, but no differences between varieties in digestibilities of specific amino acids. There were, however, large differences between amino acids, with arginine having the lowest value (85.7 %) and proline and alanine the highest (100 %). From the above literature, it may be summarized that grain quality is confounded by its type and variety or hybrid.

2.3.2 Growing, harvest and drying conditions

The growing environment such as location, season, plant density and fertility of land, and post-harvest conditions, including ripeness and moisture content at harvest play a vital role in cereal grain quality. It has been reported that kernel breakage is greatest at high plant densities (Bauer and Carter, 1986; Moes and Vyn, 1988; Vyn and Moes, 1988). The quality of cereal grain depends on growing season (temperature and rainfall) and other environmental factors (e.g. soil fertility, fertilizer use) as well as post-harvest processing (van Keulen and Stol, 1991). For instance, climatic conditions can affect starch content, and granular structure, composition and distribution in the developing grain (Tester *et al.*, 1995; Tester, 1997), which could influence the nutritive value of cereal grains. In particular, the protein content of cereals is affected more by source than the starch content, and the variation depends on the supply of starch and protein precursors (Jenner *et al.*, 1991).

Comprehensive annual surveys of French cereals (Conan *et al.*, 1992; Metayer *et al.*, 1993) showed that both season and site can have large effects on starch, crude protein and AME content of wheat, triticale and rye. There were other surveys in the UK and Australia, which confirmed the effect of site of growth on starch and fibre contents of

wheat (Longstaff and McNab, 1986). Similarly, Connor *et al*. (1976) reported significant differences in the AME values of sorghum and maize due to location and year of harvest. The season, location and variety of grains can have large effects on starch, crude protein and AME of wheat, triticale and rye (Metayer *et al.*, 1993). A survey of wheat over a three-year period conducted by Choct *et al*. (1999a) found a large dissimilarity in AME value, starch, protein and soluble and insoluble NSP due to the years of harvest and geographical locations of the wheat. Other cereals, for example, maize, sorghum, barley and oats were also relatively inconsistent across seasons and sites.

Recent studies have demonstrated that the chemical composition and nutritional value of maize are inconsistent from batch to batch, resulting in considerable variation in its energy value for poultry (Cowieson, 2005). Nyirenda *et al.* (1987) provided supporting evidence of seasonal and varietal effects on crude protein, ash, starch, crude fat, free sugars, tannins and AME of sorghum. Indications are that, in maize, the degree of grain fill is partially responsible for differences in AME. This could be due to reduced starch content or a higher proportion of cell wall content in the sample or a combination of these two factors. Chemical composition and nutritional value of grains may also vary due to the stage of maturity at harvest. On the other hand, hens were unaffected by ripeness, but weight gain and feed conversion efficiency of broilers were reduced by early harvest of barley (Thomke, 1972). It is obvious that the stage of ripeness will affect the composition of the grain, including NSP content, with the nutritive value of the grain for poultry being influenced accordingly. McNab (1991) reported that application of nitrogen fertilizer increases the total amino acids in wheat and that fungicides can have the opposite effect. Inadequate research, however, has been undertaken concerning the role of source and the agronomic practices involved in producing maize grain. The importance of environmental conditions during growth of cereal grains needs to be studied further in relation to the occurrence of low ME wheat, the variable responses to other grains by poultry, and the underlying causes of these phenomena.

Post-harvest drying and storage period

Maize is usually harvested at relatively high moisture content to minimize damage due to natural drying in the field. The background was that leaving the maize in the field for some time after physiological maturity resulted in increased losses from birds and rodents, lodging by lepidopteron stem borers and field infestation by maize weevils (Agbaka, 1996), subsequent aflatoxin contamination and reduced grain quality (Hell *et al.*, 1996), and severe grain losses after eight months storage (Borgemeister *et al.*, 1998). For example, in the Midwest, US, harvesting maize with high moisture content and drying of high moisture maize to reduce mould infection and the risk of aflatoxin contamination is the most common method of conditioning it for storage and marketing (Borgemeister *et al.*, 1998). Artificial drying of maize can cause two types of damage such as puffiness and discoloration, which will affect the efficiency of dry milling and other processes (Paulsen and Hill, 1985). Rapid drying causes brittleness, which is manifested in the form of stress cracks, leading to breakage.

The high moisture content associated with early harvest necessitated the use of highly ventilated cribs, allowing the maize to reach safe moisture content shortly after the beginning of storage. Post-harvest change in the nutritive value of wheat (Wiseman and McNab, 1997) and barley (Fuente *et al.*, 1998) has been reported to occur in Europe. Singh *et al.*(1998) indicated that there was a hybrid-dependent effect of high-temperature drying and harvest moisture content at 80 ^{o}C air drying temperature. Drying is used to maintain the condition of recently harvested, wet grain before it can be stored or further processed. Effective post-harvest grain quality management in general involves three sets of control objectives, drying (initial reduction in moisture content), conditioning (making the moisture content uniform) and aeration (lowering the grain temperature. During the drying phase, the moisture content of the grain is reduced as quickly and efficiently as possible to levels that are safer for storage. Dirk (2000) mentioned that successful transition from the drying to conditioning and aeration phases requires a keen operator who regularly monitors the progress of the drying, conditioning and temperature fronts in the grain mass. It also requires an automated and flexible fan and heater control system, which is well understood by the operator.

There is a widely held view amongst commercial feed millers that new season grains, particularly wheat and barley, are less nutritious than stored grain for poultry. Studies of Australian wheats and barleys (Choct, 1995; Choct and Hughes, 1997) indicate that AME can rise substantially within three to six months after harvest. Not all samples exhibit this phenomenon, however. Many samples have relatively high AME values immediately post-harvest and do not improve with time, whereas a few samples can remain low for up to two years (Choct, 1995; Choct *et al.*, 1995; Choct and Hughes, 1997; Hughes and Choct, 1997). The underlying basis for this phenomenon is yet to be defined. However, it is possible that a gradual *in situ* degradation of anti-nutritive substrates such as NSP may occur as grains contain endogenous enzymes.

Commonly, it is recommended that grain temperatures be maintained within 5 to10 °C of the average ambient temperature for the geographic location of the storage, to minimize the potential for convection currents (Maier, 2004). Harvest temperature varies widely for grain and seed crops across the world. In temperate latitudes, grain is generally harvested later and can be stored at higher moisture levels than in tropical latitudes. For instance, maize harvest in the southern US typically occurs from mid-July through September at moisture contents of 15 – 20 % (wet basis), but in the northern US, harvest is usually in October and November at moistures of 20 – 30 % of wet basis. That is why producers and elevator operators in the north can cool grain much sooner after harvest and artificial drying than those in the south, who generally do not have to dry artificially.

Artificial drying of maize can cause several types of damage. Rapid drying causes brittleness (Symons and Shahin, 2008). This is the most prevalent damage and is manifested in the form of stress cracks, leading to breakage. Stress cracks directly affect the ability of millers to salvage intact endosperms, and generally reduce the number of large premium grits produced in dry milling. Stress cracks also contribute to the breakage in maize during its handling. Scorching and discoloration of maize characterize damage caused by overheating. This indirectly contributes to the brittleness of the dried grain. Heat damage caused by excessive drying temperatures not only results in physical damage to the kernel that affects milling properties, but also causes undesirable chemical changes for instance, changes to starch components (amylose and

amylopectin, resistant starch), protein, minerals and NSP contents (Iji *et al.*, 2003; Svihus *et al.*, 2005). The second factor is pest activity in storage. Granary weevil (*Sitophilus granarius* L.) is the most significant infester of storage grain in Europe. The activity of insects leads to an increase in the moisture content and temperature of stored grain, which leads to conditions favourable for infection by fungi and subsequent reduction in quality by mycotoxins. Infested grain is hazardous for humans and animals due to the contamination by metabolites, body fragments, and dead pests, which are strongly allergenic to animals.

Storage conditions

Cereal grains can be stored for long periods without microbial spoilage; however, biochemical changes do occur during storage. The grain respires, dry matter is lost and functional and nutritional aspects of grain are altered (Reed, 1992). Most maize in the United States is harvested at 22 – 30 % moisture and must be dried to 15.5 % moisture or less for safe storage to prevent microbial growth. Improper natural or artificial drying has been linked to decreased starch yield from wet milling, decreased maize grit yield, increased stress cracking and increased kernel breakage (Peplinski *et al.*, 1989; Eckhoff and Tso, 1991; Peplinski *et al.*, 1994). Sokola (1991) reported on loss of nitrogen (as ammonia) and vitamins A and E, and increased fat rancidity in grains including wheat, barley, triticale, maize, faba beans, soybean and rapeseed stored for 12 months at two moisture levels. Similarly, Svihus *et al.* (1995) observed that moisture content of ensiled, rolled barley affected nutritive value and subsequent performance of broilers. Protein, amino acids, β-glucans and viscosity were reduced if barley was stored moist compared with drying to 87 % DM, and feed conversion and excreta condition were improved. In addition, Alumot *et al* (1972) warned that grain fumigants such as ethylene dibromide and ethylene dichloride can persist in cereals for several months and have adverse effects on performance of laying hens fed diets containing the fumigated grain. These reports indicate that care needs to be taken in collecting information on the use of pesticides pre- and post-harvest on grain for animal feeding. While these storage conditions are extreme compared with those normally practised in Australia, nevertheless these results point to the need to minimise the effects of storage conditions on grains for feeding.

2.4 THE EFFECTS OF PROCESSING OF CEREAL GRAINS

2.4.1 Physical quality

The modern technology of harvesting and threshing requires knowledge of the mechanical properties of cereals, if the objective is to optimise these processes and to reduce the quantitative and qualitative losses of grain. Preliminary research in the domain of cereal grain properties covered the question of the origin of quantitative losses of grain in combine harvesting. One of the factors causing these losses is the spontaneous shedding of grain in the field when cobs hit the elements of the harvesting system. Another one is the unthreshed grain left in ears. In the first case, the grains are not firmly attached to the ear torus, while in the second situation the grain is attached too securely within the ear. Improper regulation of the combine harvester can also lead to these problems (Reznicek, 1970; Reznicek *et al.*, 1978; Szot and Reznicek, 1984).

The physical quality of grain is of great importance and may enhance its value in terms of poultry feed. Dibner (1997) has shown that development of the digestive tract post-hatch, is influenced by ingredient texture and is important to broiler health. Gut development and function is becoming an important focus in feed particle size studies and is of key importance in gut health. MacMasters *et al.* (1959) opined that maize varieties grown in different seasons differed in response to drying conditions, and suggested that these responses resulted from differences in chemical composition and the physical condition of kernels. Large variation in chemical composition has been observed due to the intensity of artificial drying of different hybrids of maize (Stroshine *et al.*, 1981).

2.4.2 Chemical composition

Cereal grains are the most important sources of energy, protein, lipids and minerals for animals raised in intensive production systems. The amount of these nutrients can vary widely between both grain and animal species. In addition, the chemical compositions of grains are also affected by the post-harvest processing of grains.

2.4.2.1 Starch

Starch is an important energy source for broiler chickens. It supplies more than 50 % of the metabolizable energy in practical broiler diets. Starch is composed of amylose and amylopectin. Amylose consists of long chains of α (1⟶4) linked glucose molecules that form a tight helical structure (Figure 2.4) due to the bond angles between the glucose units, and they are relatively inaccessible to amylases, whereas amylopectin contains some α (1⟶6) linkages (Figure 2.5) that produce branches (on C_6) in the molecule and provide an open structure more readily attacked by digestive enzymes. The amylose content in normal maize starch ranges from 25 to 30 % but can be increased due to artificial drying (Brown, 1996; Iji *et al.*, 2006). That is why high amylose starches are poorly digested compared with starch containing mainly amylopectin (Black, 2001). The starches of cereal grains are highly digestible by the amylolytic enzymes of monogastric animals as they contain very little resistant starch. The properties of starch from normal cereals and high-amylose maize are variable. The drop in starch yields was from 5.8 to 18.2 % when the hybrids were harvested at high moisture contents and dried at 110 °C (Singh *et al.*, 1998).

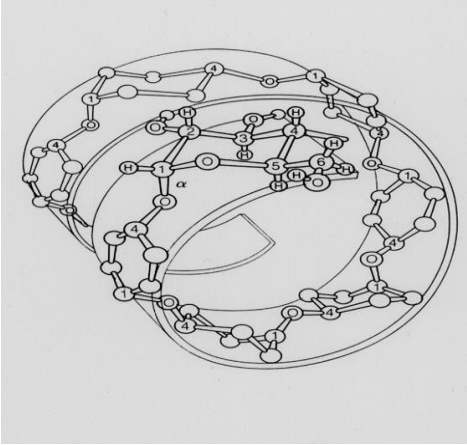
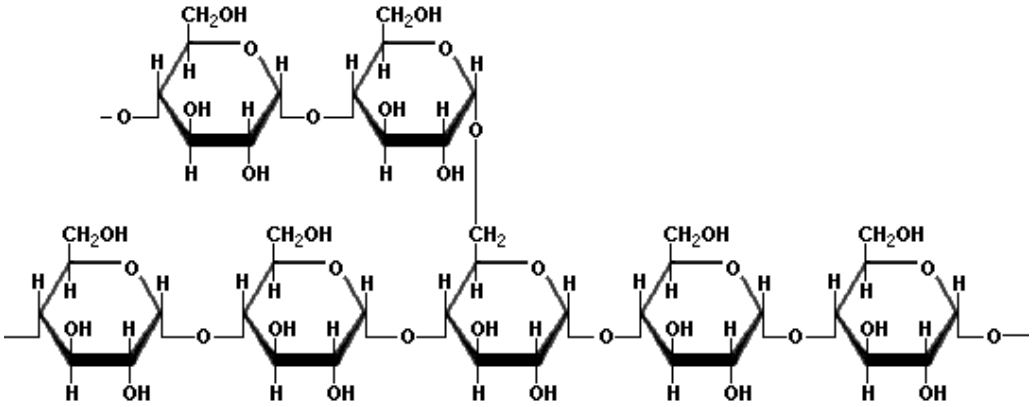


Figure 2.4 Helical structure of amylose [Adapted from Mathews and van Holde: Biochemistry 2/e@ The Benjamin/Cummings Publishing Co. Inc.]



Amylopectin

The side branching chains are clustered together within the amylopectin

Figure 2.5 Chemical structure (top) and side branching chains (bottom) of amylopectin

It is well established that starches high in amylose are poorly digestible by amylolytic enzymes and consequently act like fibre in the small intestine and are not well utilized by monogastric animals (Annison and Topping, 1994). When starch granules are fully gelatinized and dispersed, the starch becomes easily digestible. Pettersson and Lindberg (1997) found a considerably higher digestibility of starch in the small intestine when animals were fed amylopectin-rich barley (9:91, amylose:amylopectin) compared with conventional barley (30:70, amylose: amylopectin) in pigs. It can be speculated that the concentration of amylose and amylopectin can vary due to grain type and post-harvest drying with their proportion indicating the quality/nutritional value of starch as well.

2.4.2.2 Protein

The grain drying process can affect the digestion of maize in broiler chickens by damaging the structure of the grain protein, resulting in greater cohesion between starch granules and the protein matrix, which reduces starch hydrolysis by limiting enzyme

accessibility (Kaczmarek *et al.*, 2007). That is why the temperature and duration of processing has to be carefully controlled when heat treatment is necessary. Excessive heating can result in the reduction of protein solubility and may even destroy or reduce the availability of certain amino acids, especially lysine. The availability of lysine in heat-treated peas decreased by 21 % when the heating temperature was increased from 110 to 150 °C for 15 min (van Barneveld *et al.*, 1993). However, the damage to proteins that are heated in the absence of carbohydrate has also been studied (Varnish and Carpenter, 1975a, 1975b) and it has been found that cross-linkages in proteins can also occur with the amide groups of asparagine and glutamine during severe heat treatment. The influence of temperature and duration of heat treatment on the nutritional value of oil seeds has been well documented (Zhang and Parsons, 1994; Perilla *et al.*, 1997) and guidelines exist regarding the optimum processing conditions. On the other hand, when it comes to cereal grains and other legumes that are not readily heat-treated, very little information exists.

Furthermore, the biological response to heat treatment will depend on the composition of the feedstuffs as well as the presence of heat-labile ANFs. The extended steam heating of *Phaseolus vulgaris* beans from 40 to 80 min, resulted in lower weight gains and higher feed conversion ratios in piglets (Van der Poel, 1990). It is therefore important to determine the exact conditions of heating for a specific feedstuff that will not reduce the nutritive value.

2.4.2.3 Interaction between carbohydrates and proteins in cereals

With prolonged elevated heating, basic amino acids, for instance, lysine, undergo a Maillard reaction that reduces their digestibility and biological availability. This process is characterized by the non-enzymatic browning reactions that occur between the amino group of lysine and the carbonyl groups of reducing sugars such as the oligosaccharides raffinose and starch (Parsons *et al.*, 1992). In particular, when grain is introduced to a hot dry oven, a complex chemical reaction occurs on the surface. The carbon molecules contained in the sugar, or carbohydrate, combine with the amino acids of the proteins. This combination cannot occur without the additional heat source. Consequently, the heat treatment of feedstuffs such as maize with a high content of these sugars needs to

be monitored carefully. The end result of this chemical recombination is called a Maillard reaction (Mian, 2000). Leeson *et al.* (1993) found that available lysine was variable between cultivars, a situation which probably reflects the variation in heating condition imposed during drying.

2.4.3 Physical processing of grain

Overall, processing of feed grains plays an integral role in determining the nutritive benefits gained by the animal such as nutritive value and particularly energy utilization. The success of a feeding method such as mash feeding is also greatly influenced by the mode in which the grain has been initially processed. Thus it is not only important to address the physiological development of the animal when formulating a diet but also the source of grain, the mode in which it is processed during the milling phase as well as the coarseness of its particles. There are several methods used in grain processing, but the most commonly used methods are the hammer and roller milling. It has been recognized for quite some time that grinding feed by either of the above-mentioned methods does a great deal towards improving the nutritional value of the feed by altering the physical makeup of components of the grain, for example, starch (Little, 1997). However, the magnitude of such improvement is largely dependent on the particle size of the processed feed. It has also been suggested that the particle size of the mash is another critical factor in chicken diet.

Milling and grinding of grains serves the purpose of physical disruption to the cellular structure of starch granules, thereby increasing the surface area for exposure to digestive enzymes. A combination of heat and moisture treatments by steam pelleting, expansion or extrusion is used to gelatinize starch to improve its digestibility (Moran, 1982b). These processes are also likely to degrade or inactivate anti-nutritive factors such as α-amylase inhibitors (Pusztai *et al.*, 1995), more of which will be discussed in subsequent sections. On the other hand, heat and moisture treatment can result in the formation of resistant starch (Blakeney, 1993), and solubilisation of NSP (Vranjes *et al.*, 1994), which can have adverse effects on animal nutrition. The benefits of physical processing of grains for monogastric diets are debatable and need further assessment. Limited research, however, has been carried out regarding the optimum particle size for different

grains. In addition, it has not been easy to come to any definite conclusions regarding grain particle size reduction for poultry.

2.4.3.1 Milling

Milling is a critical factor for poultry feed, especially the particle size and grinding method. The efficacy of hammer milling versus roller milling greatly influences the performance of chickens (Nir *et al.*, 1994a; Nir *et al.*, 1994b; Nir *et al.*, 1995). These methods, while not identical, are grouped together since the method of action is to break the seed coat and reduce particle size. Roller milling can be used to crack the seed coat in order to allow entry of bacterial and digestive enzymes while retaining a large particle size, which partially limits the rate and extent of digestion or fermentation. Grinding or milling, conversely, can produce extremely fine particles, which can be rapidly fermented or digested. The hardness and viscosity of the grain affect its response to physical processing. The harder the grain, the more damage occurs to the starch granules during processing (Rowe *et al.*, 1999). Traditionally, the hammer mill and roller mill have been used to reduce the particle size of the grains, while the latter has been applied mainly to produce coarser feeds (Koch, 1996; Waldroup, 1997). Roller mills can, however, produce fine particles of similar uniformity to those produced by hammer mills. In either case a range of particle sizes is produced depending on a number of factors including the type of grain used, the speed of grinding and screen size used (McCracken, 2002) (Table 2.4). In the case of hammer milling, the size and spectrum of particle yield depend on the screen size and the hammer speed (Koch, 1996). The efficiency of the hammer mill is influenced by grain type, grain moisture content, screen size, feed rate, power of the motor and speed of air flow through the mill (Martin, 1985). Hammer mill grinding produces a greater quantity of fine particles (Reece *et al.*, 1985), which may confound results.

The importance of physical aspects of the diet in augmenting digestion and productivity is increasingly recognized in the broiler industry. Feed ingredients based on seeds such as cereal grains and grain legumes are subjected to some type of particle size reduction prior to incorporation into poultry diets. No significant effect on broiler performance was found due to grinding grains through a hammer and roller mill, when diets of

similar geometric mean particle diameter were compared (Nir *et al.*, 1990). Conversely, Douglas *et al.* (1990) observed that significantly greater body weights and better feed conversion were observed in chickens fed diets containing grains ground through a hammer mill only when compared to grains ground by both a hammer mill and a roller mill.

Table 2.4 Milling output of different grains[1]

Grain	GMD (μm)	GSD	Reference
Hammer mill			
Maize	947	2.07	Douglas *et al.* (1990)
Sorghum	841	1.77	
Sorghum	628	1.88	Nir *et al.* (1995)
Wheat	681	2.29	
Maize	890	2.19	Amerah *et al.* (2008b)
Wheat	528	2.33	
Roller mill			
Maize	1470	1.82	Douglas *et al.* (1990)
Sorghum	1800	1.40	
Maize	1413	1.76	Nir *et al.* (1995)
Sorghum	2170	1.65	

[1]In each study, the grains were ground in the same mill under similar conditions;
GMD = Geometric mean diameter; GSD = Geometric standard deviation.

Evidence suggests that, during grinding (hammer or roller), different screen sizes may have to be used according to the grain type to obtain the desired particle size distribution. However, it must be noted that even within a grain type, grinding in the same mill type under similar conditions may result in different particle sizes due to variations in endosperm hardness. Lentle *et al.* (2006) acknowledged that grinding grains from three cultivars of wheat in a hammer mill through the same sieve resulted in different particle size distributions. While feed mills have become larger and throughput has increased, there seems to have been less of an emphasis on feed quality and, more specifically, physical structure (Beyer, 2003). This is surprising, considering that properly formed feed can result in saving more feed conversion points than many other strategies. Beyer (2003) also mentions that particle size is another manufacturing parameter in the feed mill industry needing close attention.

The physical form of the grains used in poultry diets can have a bearing on the nutritive value, particularly energy utilization. Milling and grinding of grains served the purpose

of physical disruption to the cellular structure of starch granules, thereby increasing the surface area for exposure to digestive enzymes. The influence of particle size appears to be confounded by the complexity of the diet and nature of feed processing, such as milling (hammer mill versus roller mill), pelleting and crumbling (Goodband *et al.*, 2002). Furthermore, it would appear that the grain type also influences the responses to feed particle size differences. Feed particle size has a pronounced effect on several physiological characteristics of the gastrointestinal tract of broiler chickens (Engberg *et al.*, 2002). Other benefits of particle size reduction include a generally greater ease of handling and mixing of ingredients (Koch, 1996). The benefits of physical processing of grain for monogastric diets are debatable and need further assessment. However, when diets were fed in crumbled form, performance was similar between the two processing systems and superior to that of chicks fed the mash diets.

2.4.3.2 Pelleting

The structure of feed for broiler chickens has a strong influence on the physiological functions of the digestive tract. Most feed used for commercial industrial broilers is heated and compressed to form pellets. Pelleting is associated with positive effects for both feed handling and bird performance, including increased feed utilization and better growth rate (Leeson and Summers, 1991; Gibson, 1995; Jensen, 2000). It is generally accepted that, compared to mash, the feeding of pellets increases feed intake and improves broiler growth (Nir *et al.*, 1994a; Nir *et al.*, 1995). Most other authors have also observed an improved feed conversion rate with pellet feeding. This may be explained by the higher digestibility of nutrients, since granulation may expose the feed particles more efficiently to further enzymatic digestion (Calet, 1965). Pelleting can also be associated with negative effects, particularly when the mixture is over-heated. Overheating can result in denaturation of protein (Araba and Dale, 1990) and resistant starch (Blakeney, 1993; Brown, 1996) to digestion, solubilization of NSP (Vranjes^ *et al.*, 1994), inactivation of vitamins (Pickford, 1992) increased intestinal viscosity (Nissinen, 1994) and inactivation of endogenous enzymes (Inborr and Bedford, 1994; Pusztai *et al.*, 1995). Samarasinghe *et al.* (2000) have revealed that pelleting at temperatures as high as 90 °C drastically reduces cellulase activity, energy and nutrition utilization, thus lowering broiler performance.

2.4.3.3 Particle size and grain quality

It is generally thought that smaller particles with an increased surface area will allow increased access to digestive enzymes and enhance digestion of nutrients (Waldroup, 1997). The influence of diet particle size appears to be confounded by the complexity of the diet, type of grain (Amerah *et al.*, 2007), type of mill used (hammer mill versus roller mill) and further processing such as pelleting or crumbling (Goodband *et al.*, 2002). It has been suggested that coarse feed particle size would be beneficial to improved feed efficiency in broilers fed wheat-based diets (Lentle *et al.*, 2006) .

The percentage of fine particles obtained after grinding depends on the hardness of the grain, with a higher percentage of fine particles from softer grains (Carre *et al.*, 2005). A harder endosperm gives larger particles with more irregular shapes, while a soft endosperm produces smaller sized particles (Rose *et al.*, 2001). This effect may be responsible for the better broiler performance reported with mash diet based on hard wheat (Rose *et al.*, 2001; Pirgozliev *et al.*, 2003). Endosperm hardness in wheat cultivars is known to influence the milling outcome, but not much is known about the effect of milling in maize.

Uniformity of particle size also appears to influence performance when mash diets are fed, and best performance was obtained using diets incorporating medium (dietary GMD of 769 μm) maize (Nir *et al.*, 1994c). Consistency of the diet has long been assumed to be important for optimum performance of broilers, especially those raised in close confinement using automated feeding equipment. McCoy *et al.* (1994) demonstrated the importance of a low coefficient of variation on average daily gain and feed conversion by growing chicks. Maize particle size also significantly affects the utilization of Ca, total P and phytate P and the greater utilization of these nutrients is found in coarser, rather than fine particle diet (Kasim and Edwards Jr, 2000). Therefore, possible implications of particle size and diet uniformity are of importance when considering fineness of grinding.

Feeding broiler chickens with finely ground wheat grain in the early growing period is not recommended due to the increased ileal viscosity and depressed performance, although the negative effects of finely milled wheat grain were overcome during the

finishing period even with 50 % of wheat inclusion in diet. Medium and coarsely milled wheat grain remain the preferred form of cereal grains, although whole grain is of great importance considering that there is no grinding cost (Yasar, 2003). The above literature indicates that grain particle size is very important in terms of bird performance.

2.5 EFFECT OF GRAIN PROCESSING ON NUTRITIVE VALUE

Cereals have to be processed in order to maximise the bioavailability of nutrients before feeding to chickens. The bioavailability of nutrients can be best defined as the amount of nutrient present in a diet, which is eventually absorbed by the GI tract.

2.5.1 Feed consumption

The amount of a diet consumed by animals depends primarily on the requirements for nutrients to meet metabolic demand, the volume of the digestive tract and the rate of passage of digesta through the tract (Forbes, 2005). Modern broiler strains have been selected for appetite, although the physiological basis for feed intake regulation is not clearly understood. Portella *et al.*(1988) suggested that birds eat smaller particle sizes at the end of a 24h period, especially when the proportion of large particles was reduced. Chemical analysis reveals that birds selected material on the basis of particle size, since nutrient composition of the diet and of different diet fractions did not change throughout the day. Lentle *et al.* (2006), who conducted research on wheat-based diets, mentioned that coarse feed particle size is advantageous in terms of feed efficiency in broilers. Significantly greater body weights and better feed conversion were attained in chickens on diets containing grains ground by a hammer mill compared to those ground by a roller mill (Douglas *et al.*, 1990). It has also been reported that feed intake is greatly influenced by the grain type and particle size in the diet and it is speculated that there is a comparatively feed intake of diet containing fine particles compared to diet containing coarse particles (Table 2.5).

2.5.2 Growth response

Growth is the main production target for broiler chickens. However, growth rate depends on various factors such as grain processing and particle size, grain and diet quality. Yasar (2003) opined that diets based on fine wheat particles can cause depressed performance in broiler chickens from day 0 to 28, during which time ileal digesta viscosity increased, but by day 42 this was nearly compensated for by an increased growth rate and a return to normal gut size. In particular, in maize-based diets, coarse grinding improved weight gain, when compared to fine grinding (Amerah *et al.*, 2008b). It was postulated by Reece *et al.* (1985) that coarsely ground maize increases body weight and improves feed efficiency of broilers (Table 2.5).

Table 2.5 Effect of particle size on the performance of broilers fed mash diets

Grain	Age (days)	Particle size (μm)	Feed intake (g/bird)	Gain (g/bird)	FCR (g/g)	Reference
Maize	1-21	814	-	582	1.43	Reece *et al.* (1985)
		1343	-	635	1.40	
Maize	1-21	947	-	521	1.49	Douglas *et al.* (1990)
		1470	-	488	1.55	
Maize	7-21	897	725	522	1.37	Nir *et al.* (1994c)
		1102	716	463	1.54	
		2010	740	473	1.60	
Maize	1-21	Fine	1191	823	1.45	Amerah *et al.* (2008b)
		Coarse	1173	870	1.36	
Wheat	1-21	Medium	1102	810	1.37	Amerah *et al.* (2008a)
		Coarse	1079	786	1.37	
Wheat	1-21	Fine	1357	888	1.53	Amerah *et al.* (2008b)
		Coarse	1262	872	1.47	
Sorghum	7-21	Fine	532	364	1.46	Nir *et al.* (1990)
		Medium	548	376	1.46	
		Coarse	561	382	1.47	
Maize and wheat	7-42	Fine	-	1942	1.91	Hamilton & Proudfoot (1995)
		Medium	-	1982	1.92	
		Coarse	-	2004	1.92	
Maize wheat and sorghum	1-21	Fine	591	357	1.65	Nir *et al.* (1994b)
		Medium	662	427	1.55	
		Coarse	645	401	1.60	

In addition, higher gizzard weights (Amerah *et al.*, 2008b) and a lower gizzard pH were found in broiler chickens with coarsely ground grains (Nir *et al.*, 1994c). A low pH in

the glandular stomach of monogastric animals favours the weak acid groups of phytate and reduces the formation of phytase-resistant mineral-bound complexes (Bedford and Schulze, 1998). It was postulated by Reece *et al*. (1985) that chickens on a mash diet containing maize ground with a roller mill had superior weight gain and feed conversion ratio compared to chickens fed on a mash diet containing maize ground in a hammer mill. Moreover, evidence suggests that the large grain particle size influences broiler performance to a greater extent when birds are fed mash diets than when fed pelleted or crumbled diets (Nir and Ptichi, 2001; Amerah *et al.*, 2007).

Promotion of growth of the gizzard is one nutritional strategy which can be achieved by manipulating feed particle size (Nir *et al.*, 1995; Engberg *et al.*, 2002). A well developed gizzard is associated with improvement in gut motility (Ferket, 2000) and may prevent pathogenic bacteria from entering the small intestine (Bjerrum *et al.*, 2005), thus reducing the risk of coccidiosis and other enteric diseases (Engberg *et al.*, 2004; Bjerrum *et al.*, 2005). Part of the beneficial effects of feed particle size may arise from their influence on intestinal morphometry, but there are no published data on this relationship. Data on the effect of feed particle size on the particle size distribution of digesta in poultry are also scanty. Lentle (2005) has speculated that greater proportions of coarser particles transiting the gizzard may increase the permeability of digesta to enzymes and improve digestive efficiency. Greater development of the broiler gastrointestinal tract suggests that feed may be retained in the upper digestive tract for a longer period, allowing for increased enzymatic digestion (Hetland *et al.*, 2002).

It is generally thought that smaller particle size with an increased surface area improves access for digestive enzymes for the digestion of nutrients (Waldroup, 1997) and presumably lower energy expenditure on mastication (Jurgens, 1993). However, if the particle size of the grain is too small, it results in a decrease in broiler performance. On the other hand, a diet ground too coarsely is also inefficiently utilized due to the rapid passage of the large particles through the digestive tract resulting in ineffective or incomplete mastication and digestion of the diet (Ivan *et al.*, 1974). This suggests that the optimum particle size for hammer and roller milling becomes critical for effective feed processing.

2.5.3 Nutrient digestibility

Starch is the primary nutrient in cereal grains and its digestibility is often considered to be more than 95 %. However, several authors have reported incomplete starch digestion of cereal and legume grains in broiler chickens (Rogel *et al.*, 1987; Yutste *et al.*, 1991; Weurding *et al.*, 2001). Moreover, results from digestibility studies by Yutste *et al.* (1991) and Weurding *et al.*(2001) found considerable differences between sites and the rate of starch digestion between feedstuffs. Enting *et al.* (2005) also illustrated a considerable difference in starch digestion rate depending on the processing of the starch sources used, and also differences in amino acid levels obtained by the addition of casein and glutamine. Therefore, it may be important to take starch digestion characteristics into account when formulating broiler chicken feeds. Pelleting increased performance, and increased apparent metabolisable energy (AME) of the diets from 11.6 to 11.8 MJ/kg (Svihus *et al.*, 2004). However, the increase in AME was not reflected in a higher starch digestibility. On the other hand, Cowieson (2005) also emphasized the importance of phytate content, as it may affect starch digestion through the formation of tertiary complexes with starch or binary complexes with calcium, a cofactor required for alpha amylase activity. In wheat, it may be dependent in part on the level of NSP, primarily pentosans, which are a constituent of the cell wall. Higher amounts of pentosans increase the gut viscosity, reduce the digestibility of the feed and reduce the performance of young broilers (Choct and Annison, 1990). The ME content of barley for poultry varies from 11.6 to 13.8 MJ/kg DM. This variation is mostly associated with difference in lipid digestibility due to the presence of soluble pentosans (Classen and Stevens, 1995; Choct *et al.*, 1999a).

The rate of *in vitro* enzyme digestion of starch from sorghum and maize grains declines substantially as the amylose content increases (Black, 2001). Pettersson and Lindberg (1997) showed that in pigs there was a significantly higher starch digestibility in the small intestine when amylopectin-rich barley (9:91, amylose:amylopectin) was compared with normal barley (30:70, amylose:amylopectin). Granfeldt *et al.*(1993) found that in rats that digestion in the small intestine of starch from low-amylose maize was 0.96 compared with 0.68 from high-amylose maize. The effects of particle size on overall diet digestibility are not profound in older pigs, although positive responses have

been found for sorghum in weaner pigs (Healy *et al.*, 1994) and maize in finisher pigs (Wondra *et al.*, 1995).

Processing, especially heat treatment, may contribute to the variability in utilization of nutrients in ingredients. For instance, lysine is heat-sensitive and the low digestibility of lysine in cotton seed meal may reflect heat processing of the meal. Another aspect of processing, grinding, modifies particle size and shape without causing nutrient changes in feedstuffs. It has been shown that grinding improves nutrient digestibility in birds (Hamilton and Proudfoot, 1995). This may reflect the increased surface area available for enzyme attack during digestion.

Ravindran *et al.* (1998) provided comprehensive data on digestible protein and amino acids in Australian feedstuffs and concluded that apparent digestibility values were additive, which means that supply of digestible amino acids in the complete diet could be predicted accurately from the ingredients. In contrast, Hughes *et al.* (1998) observed significant reduction in digestibility of amino acids with increasing dietary inclusion rate (100/300 g/kg) of milled whole seed of *Lupinus angustifolius* cv. Gungurru. Furthermore, digestibility coefficients differed according to whether the cereal component was sorghum, corn, or wheat. In a recent review, Williams (1995) pointed out that gut microflora can significantly influence metabolism of gut tissue, which in turn will affect absorption of amino acids. Relatively small differences between ileal and faecal digestibilities in grains observed by Williams (1995) could become significantly more important when comparing different samples of grain. It is likely that dietary factors which lead to increased activity of gut microflora will depress energy utilization (Choct *et al.*, 1996) , apparent protein digestibility (Smits *et al.*, 1997), and availability of amino acids (Steenfeldt *et al.*, 1995).

The digestibility of amino acids was affected by the protein level of the feed and by feed intake, and accurate CP and amino acid digestibility estimation should use the appropriate type of bird and diet (Angkanaporn *et al.*, 1997). Klopfenstein and Hoseney (1995) showed sorghum protein to be less digestible than protein from other cereal grains because of the presence of γ-kafirins. However, Silano (1977) reported that the digestibility of protein ranged from 30 to 70 % in different sorghum cultivars and a

recent mutant (P21N) has a digestibility of 85 % (Oria *et al.*, 2000). The digestibility of protein and starch in barley also varies, but to a lesser extent.

In a research study, McAllister *et al.* (1993) demonstrated that maize starch appears to be more resistant to rapid digestion than wheat or barley in its native form, and this may explain why maize responds so well to enzymes in the absence of any viscosity considerations. On the other hand, Kaczmarek *et al.* (2009) found an improvement in apparent digestibility of all nutrients except fat in broiler chickens fed maize-based diets with enzyme supplementation, while CP digestibility was improved by 2.9 % through enzyme supplementation (Avizyme 1500) in maize-soybean-based diets (Zanella *et al.*, 1999) in chickens. Cowieson and Ravindran (2008) reported that energy and amino acid values of maize-soybean-based diets for broilers can be enhanced by supplementation with an enzyme combination of xylanase, amylase and protease.

2.5.4 Energy utilization

Cereal grains are the major source of energy for commercial poultry and represent from 60 to 70 % of the diet. Cereal grains, combined with legumes and oilseed meals provide not only the bulk of the energy and other essential nutrients for commercial poultry production but are also the prime source of anti-nutritive components which are likely to have a significant bearing on how effectively all dietary components are utilized by poultry. However, the capacity of cereal grains to provide energy to birds varies widely between and within grain species. This variation in energy value of grains due to soluble NSP stands out as a major determinant of the availability of energy and other nutrients for poultry (Hughes and Choct, 1999). The amount of energy supplied by a grain depends on both the extent of digestion and the amount eaten. The extent of digestion depends on the adequacy of enzymes within the digestive tract capable of breaking specific chemical bonds in each grain component. Accessibility of the enzymes to the chemical components depends on time retention, and enzymes and components are associated. Much of the variation between grains in energy digestibility is explained by differences in gross chemical composition (Black, 2001). However, other factors, particularly those that affect the accessibility of enzymes to specific grain components can markedly affect the digestibility of grains and availability of energy.

The efficiency with which available energy is utilized for chicken growth or egg production depends on its synchronous availability for metabolism in body tissue with amino acids and other essential compounds. It is likely that dietary factors which lead to increased activity of gut microflora will depress energy utilization (Choct *et al.*, 1996), apparent protein digestibility (Smits *et al.*, 1997), and availability of amino acids (Steenfeldt *et al.*, 1995).

Wheat variety had a greater impact on AME than either production site or harvest year (Wiseman and McNab, 1997). In contrast, Choct (1995) found that seasonal effects had a greater influence than variety on the AME and NSP content of wheat varieties from mainland states in Australia. The incidence of 'low-ME' wheat varieties with a high soluble NSP content was linked to rainfall and environmental temperature patterns during the period of grain maturation. Supporting evidence has come from observed differences in AME of the same varieties of wheat grown on the same site in different seasons (Hughes, unpublished data). It appears that hot, dry conditions may increase the proportion of cell wall material that is high in soluble NSP content and consequently reduce the AME value of the grain (Aastrup, 1979).

2.6 ENZYME SUPPLEMENTATION OF DIETS

The main objective of adding exogenous enzymes to poultry diets is to improve the utilization of nutrients in raw materials (Bedford and Schulze, 1998). This can be achieved by one or more of the following mechanisms: degradation of specific bonds in ingredients not usually degraded by endogenous digestive enzymes, degradation of anti-nutritive factors that lower the availability of nutrients, increased accessibility of nutrients to endogenous digestive enzymes, and/or supplementation of the enzyme to enhance the capacity of young animals. The enzymes widely used by the industry are the carbohydrases (xylanases and β-glucanases) that reduce the NSP in some cereals and, more recently, microbial phytases that target the phytate complexes in plant derived ingredients. The effects of xylanases and β-glucanases are well known (Annison and Choct 1991; Bedford and Schulze, 1998), but the corresponding information on the influence of enzyme mixtures with microbial phytase is limited.

Enzyme supplements used in poultry diets have a straight, positive outcome on animal performance by aiding in the absorption of nutrients and by reducing the viscosity of digesta caused by NSP fractions present in various cereals (Danicke *et al.*, 1999). In this regard, enzymes have been reported to improve bird performance, presumably by increasing nutrient digestibility and utilization (Hesselman *et al.*, 1982). The roles of exogenous enzymes in improving the nutritive value of grains and in reducing the problems associated with wet, sticky droppings have been reviewed extensively by Bedford and Morgan (1996) , and Williams (1997). Enzymes are thought to act on two fronts. The first action is to reduce viscosity of digesta by partial depolymerization of main and side chains in complex carbohydrates such as arabinoxylans and β-glucans in cereals. The second action involves disruption of the cell walls to expose substrates such as starch to digestive enzymes. It has been pointed out that the reduction of digesta viscosity by exogenous enzymes does not fully account for production responses in broilers fed barley-based diets (Chesson, 1993). This researcher mentioned that the release of nutrients following enzyme degradation of cell walls was an important factor. Bedford and Morgan (1996) concluded that digesta viscosity was the major factor limiting performance of broilers in the case of wheat-based diets. Enzyme supplementation of broiler diets improves the digestibility of some dietary components, such as fibre and starch (Bedford and Schulze, 1998). Heat processing of cereals gelatinizes starch to some extent (Medel *et al.*, 2000), facilitating endogenous enzymatic degradation, and solubilizes fibre components that might enhance the activity of exogenous enzymes (Vukic-Vranjes and Wenk, 1995).

Another important anti-nutritional factor present in feedstuffs is phytic acid, which binds minerals, proteins, lipids and starch (Thompson and Yoon, 1984) and reduces the digestibility of these nutrients for poultry (Sebastiana *et al.*, 1997). The role of microbial phytase supplements in poultry diets in increasing calcium and phosphorus availability has been well established in the scientific literature. Studies have also shown a beneficial effect of phytase addition (1200 FTU/kg) to broiler diets on ileal digestibility of metabolizable energy and total amino acids (Namkung and Leeson, 1999; Ravindran *et al.*, 1999).

In particular, there are two main modes of action that have been proposed for xylanase and β-glucanase in wheat- or barley-based diets; the viscosity and cage effect (Bedford, 2002). The viscosity theory suggests that as the incremental performance enhancement associated with the addition of these enzymes to poultry diet is beyond that which can be explained by the nutritive value of released sugars, there must be an additional advantage conferred by the enzymes to the digestibility of the nutrients in the diet. As high molecular weight soluble arabinoxylans and mixed linked β-glucans have a high affinity for water, they have the ability to stimulate an exponential increase in the viscosity of the contents within the GI tract (Nilsson *et al.*, 2000). High intestinal viscosity is negatively correlated with animal performance and nutrient digestibility, and is also associated with detrimental changes in the microbial flora within the distal GI tract. Therefore, facilitating a reduction in viscosity with exogenous enzymes confers a nutritional advantage to the animal (Adeola and Bedford, 2005; Cowieson *et al.*, 2005a; Sieo *et al.*, 2005). The negative effects of viscosity on animal performance are so pronounced that it has been suggested that the viscosity of feed ingredients can be used as a reliable indicator of the nutritional value of the ingredient and the efficacy of exogenous pentosanases (Bedford and Classen, 1993).

Furthermore, the effects of the viscosity of a diet are more pronounced in young animals than in older birds, presumably associated with the maturity of the GI tract in older birds and the capacity to cope with soluble polysaccharides (Yasar and Forbes, 2000). The cage effect is associated with the effects of carbohydrases on cell walls, reducing their integrity and thus releasing nutrients that were previously encapsulated (Bedford, 2002). It is likely that both mechanisms are involved in the responses to xylanase and β-glucanase in poultry diets, leading to beneficial changes in performance, nutrient retention, a reduction in incidence of sticky droppings and wet litter, and desirable changes in the microflora in the distal GI tract (Choct *et al.*, 2004; Shakouri and Kermanshahi, 2005). Currently, research in the area of carbohydrases for diets based on wheat and barley is focused on more detailed mode of action studies, the effects of enzymes for species other than broilers, assessing the importance of enzyme inhibitors and resistant starches, and also processing effects such as particle size, conditioning temperature and feeding of whole grain.

One interesting new area of research on xylanase in wheat-based diets is the role that the molecular architecture of the enzyme plays in its affinity for soluble and insoluble fibre and the susceptibility to inhibition. For example, a recent study by Fontes *et al.* (2004) demonstrated that a xylanase with a carbohydrate-binding module was approximately 5 to 6 % more efficacious in wheat- and rye-based diets, respectively, compared with a commercial enzyme preparation that lacked the ability to attach to its substrate. The authors concluded that the ability of enzymes to bind to their substrate using attachment modules increases the efficacy of the enzyme *in vivo*, especially when associated with the hydrolysis of insoluble carbohydrate.

The use of exogenous enzymes in diets that are based on maize is receiving considerable attention in recent research. This may be because of the size of the global maize feed market, the realization that maize is a variable feed ingredient and the relatively low degree of penetration of enzyme products into this feed segment, which is a thrust for commercially funded research programmes. A recent review (Cowieson and Adeola, 2005) summarized the positive effects of enzyme supplementation of maize-based diets and speculated some modes of action for various enzymes. An earlier review (Summers, 2001) regarding exogenous enzyme for maize is also comprehensive in identifying the factors in maize that are important in determining its nutritional value for poultry and the effects of exogenous enzymes. The susceptibility of exogenous enzymes to inhibition is also the subject of some discussion as the relevance of the concentration of xylanase inhibitors in maize is not clear.

2.6.1 Effects of enzyme supplementation on grain and diet quality

The use of enzymes in poultry feed has increased dramatically in the last decade. Zanella *et al* (1999) mentioned that enzymes are used primarily with wheat and barley in mixed feeds, but may also be useful in diets based on maize and soybean to improve nutrient digestibility and broiler performance. The positive effect of exogenous enzymes, particularly xylanase and β-glucanase on the nutritive value of wheat- and barley-based diets for poultry has been well established by comprehensive review papers by Bedford and Schulze (1998), and Acamovic (2001). However, the use of

exogenous enzymes, with the possible exception of phytase in maize-based diets, has been less commonly reported in the scientific literature.

Not much is known about the performance of poultry fed on a maize-based diet with enzymes due to the perception that maize has a relatively high and consistent nutritional value for poultry and, therefore, there is little to be gained by adding enzymes. In addition, it has been established that pelleting can have an effect on the nutritional value of poultry diets. This may be caused by the interaction between the effects of pelleting temperature and exogenous enzymes. Cowieson *et al.* (2005b) acknowledged that the performance of chicks could be improved by the addition of enzymes to the maize-based diet which was pelleted at 70 °C. The starch from maize has a high digestibility (97 %) and improved growth performance and the mortality of broilers declined with supplementation of a proper combination of enzymes such as amylase, xylanase and protease (Cafe *et al.*, 2002) as well as the addition of phytase (Cowieson *et al.*, 2006b, 2006c).

Cowieson *et al.* (2005b) observed that pelleting wheat-based diets above 80 °C can compromise bird performance and that if higher temperatures are to be employed, the use of exogenous xylanase is critical to maintain productivity. In recent years, enzyme supplementation to eliminate the anti-nutritional effects such as NSP has been an active subject of research (Bedford and Morgan, 1996) and is now standard industrial practice in many areas where cereals are used.

Phytic acid in feedstuffs acts as an anti-nutritive factor which binds minerals, proteins, lipids and starch (Thompson and Yoon, 1984) and reduces digestibility of these nutrients for poultry (Sebastiana *et al.*, 1997). However, it may be possible to increase the Ca and P availability in poultry diets by the addition of microbial phytase (Namkung and Leeson, 1999; Ravindran *et al.*, 1999). In addition, Wu *et al.*(2004) stated that microbial phytase supplementation improved apparent ileal phosphorus digestibility for maize, sorghum, wheat and barley; however, apparent metabolizable energy increased by 2.6 and 7.8 % only for maize and barley, respectively. Nonetheless, most recent studies indicate that microbial phytase only has a positive effect when the diet contains an optimum level of Ca, otherwise growth performance may restore (Powell *et al.*, 2010).

The effect as well as the net value of enzymes on nutrient requirements and the net value of energy and amino acids is a largely unexplored area. For instance, does exogenous protease have a sparing effect on endogenous protease production via a hormonally controlled feedback mechanism? If so, then it is possible that supplemental enzymes may reduce birds' requirements for energy and amino acids. This may hold true for other enzymes such as amylase, maltase, isomaltase and lipase. There is no reason to allow the birds to produce their own enzyme complements if they cannot do so more cheaply than the commercial fermentation process.

2.6.2 Changes in microbial population

A suitable gut microbiota is important for the optimal growth and performance of chickens, and several gut bacteria isolated from chickens have been shown to play various important biochemical roles. Alternatively, an unfavourable microbiota may promote clinical and sub-clinical enteric infections, leading to decreased intestinal activity and increased mortality. Necrotic enteritis is typified by intestinal lesions, diarrhoea, impaired digestive function, reduced nutrient absorption and decreased feed intake (Kocher *et al.*, 2004). Intestinal microflora includes unicellular microorganisms housed in the digestive tract, for example, bacteria, fungi, and protozoa. These populations represent a wide range of interacting metabolic and morphometric types. It is recognized that the microbial community and its activity in the broiler intestinal tract are influenced by diet composition. In this respect, the effect of different grains (Wanger and Thomas, 1978; Mathlouthi *et al.*, 2002; Apajalahti *et al.*, 2004) and also exogenous enzymes (Engberg *et al.*, 2004; Jozeflak *et al.*, 2006) have been reported. It has also been demonstrated that the anti-nutritional effects of soluble cereal NSPs for broilers is partially mediated via intestinal microbial activity (Choct *et al.*, 1996; Choct *et al.*, 1999b). In particular, the presence of viscous polysaccharides has been shown to increase the intestinal microbial activity associated with poor broiler growth performance (Choct *et al.*, 1996; Langhout *et al.*, 1999). The probabilities that gut microflora play a role in the depression of starch digestion and AME was established by Choct *et al.* (1996) and Smits *et al.* (1997). The mechanism of action of soluble NSP is thought to involve increased viscosity of digesta, which limits contact between nutrients and absorption sites on the intestinal mucosa (Annison, 1993a; Bedford and Morgan,

1996; Smits *et al.*, 1997). The gut microflora can influence metabolism of gut tissue significantly, which in turn will affect absorption of amino acids (Williams, 1995). However, the increased interest in the study of broiler GIT microflora in recent years is due to the ban on antibiotic growth promoters in diets.

In a review, Williams (1995) pointed out that gut microflora can significantly influence metabolism of gut tissue, and this will in turn affect absorption of amino acids. Protein supplements with poor digestibility will undergo more microbial fermentation than highly digestible material. For example, differences between ileal and faecal digestibilities in intact compared with caecectomised cockerels were minor for cereals and oilseeds, but were large for some animal meals. Nevertheless, relatively small differences between ileal and faecal digestibilities in grains observed by Williams (1995) could become significantly more important when comparing differences between different samples of grain. Choct *et al.* (1996) and Spring (1997) postulated that exogenous enzymes can exert indirect effects on animal health by manipulating the growth of gastrointestinal tract micro-organisms, including bacterial pathogens.

It is difficult to predict what the next leap forward will be in the field of feed enzyme research. However, there are some obvious gaps in knowledge that may lead to discoveries with significant implications. For example, the area that is largely uninvestigated is the use of enzymes as direct antimicrobials to lyses attachment polysaccharides or bacterial cell walls and the effect of enzymes on the immune competence of the host. The provision of substrates for beneficial bacteria by targeted enzymes and the potential impact on immune status is another area for research.

2.7 RELEVANCE OF CURRENT STUDY

From the literature, it is evident that a large variation exists in physical characteristics, chemical composition, and nutritional value of different cereals as well as between varieties and hybrids. This could lead to considerable inaccuracies when establishing the nutritional value of a particular cereal, for instance maize for broiler chickens. It is therefore necessary to be specific about the variety or source of grain that is used in a particular trial.

As seen from the literature covered, there are contradictory results of broiler performance associated with particle size of mash feed, when milled using a hammer or roller mill, and further research is needed in order to clarify these issues.

When dealing with poultry, however, the nutritional value of feed ingredients is more adequately described by the available nutrient content and should closely reflect broiler performance parameters such as feed intake, body weight, and feed conversion ratio. From the previous literature reviews, it is evident that there are lots of variations in performance due to variation in nutritional value of grains in diets. These variations depend on many factors such as postharvest processing, storage, milling, particle size, feed form, and feeding with or without a microbial enzyme supplement. This study will aim to investigate the effects of these factors on broiler performance especially on maize-based diets.

Postharvest processing, in particular, artificial heating of high-moisture grain is the most common practice in many areas of the world. Very few studies, however, have been conducted on the alteration in chemical composition due to heating. Nor is there much in literature about the subsequent overall effect of nutritional quality of grains in terms of broiler performance. In this study, the possible impact of artificial heat treatment of high-moisture maize grain on chick growth and feed conversion efficiency will be investigated.

Although gut microbial profiles can be altered through dietary and husbandry manipulations, exogenous microbial enzyme are likely to be more effective in maintaining the delicate balance of chicken gut microflora. Thus, there has been increased interest in the application of microbial enzyme products to enhance intestinal activity. However, some current literature suggests the use of exogenous enzyme, in particular, microbial phytase with an optimum dietary level of Ca in poultry diets. The current study will investigate this result using a microbial phytase enzyme.

The research limitation indicated in this literature review will be addressed in two Chapters (Chapters 3 and 4), which will deal with the variations in chemical composition and ultra-structure of low-moisture maize (LMM) grain from different sources with or without heat treatment, and the interactive effect of source of grain, feed

particle size and milling technique without any supplement on broiler performance. In addition, the following Chapter (Chapter 5) aims to determine the optimal inclusion level of maize grain with or without microbial enzyme supplementation in diets on the performance of broiler chickens. Furthermore, the last two experimental Chapters (Chapters 6 and 7) deal mainly with the variation in chemical composition and ultra-structure of high-moisture maize (HMM) grain from a single source and sun-dried or dried artificially at different temperatures. To explore these issues, a feeding trial was conducted to compare the effect of post-harvest drying of maize with or without enzyme supplementation on broiler performance, nutrient digestibility and microbial activity of broiler chickens.

CHAPTER 3 EFFECT OF HEAT TREATMENT AT FIXED TEMPERATURE ON THE NUTRIENT COMPOSITION AND ULTRA-STRUCTURE OF SUN-DRIED MAIZE GRAIN

3.1 INTRODUCTION

Maize (*Zea mays*) is one of the most important cereal grains used in feeding of poultry all around the world. It is also generally believed that maize has a relatively high and consistent nutritional value for broiler chicks but recent data indicate that the energy value of maize and nutrients available can vary and in the case of energy level may be greater that ± 162 kcal/kg (Song *et al.*, 2004). There are many suggested causes for this variation such as chemical composition, starch content, amylose:amylopectin ratio, starch structure, the embedding of starch with a matrix of lipid, protein or carbohydrate and the presence of amylase inhibitors and phytate concentration (Cowieson, 2005).

However, the principal anti-nutritive factors, viscous non-starch polysaccharides are relatively low in maize, but present in most temperate cereals such as wheat, triticale and barley (Bedford, 1995; Smits and Annison, 1996; Bach Knudsen, 1997). Regardless of these advantages, the nutritive value of maize is known to fluctuate widely, due to the environmental conditions during growth, harvest and post-harvest processing, especially drying temperature and storage (Leeson *et al.*, 1993; Collins *et al.*, 1998; D'Alfonso, 2002; Iji *et al.*, 2003).

Starch from grain is the main energy source for poultry and the ratio between amylose and amylopectin plays a significant role in the digestibility of maize starch. Noy & Sklan (1994) opined that about 15 % of maize starch remains undigested up to the terminal ileum and is assumed to be resistant to digestive enzymes. This is known as resistant starch (RS3). It escapes digestion in the small intestine and has dietary-fibre like properties. There is potential for use of exogenous enzymes to digest resistant starch and increase overall digestibility of maize as is practised with wheat and other temperate cereals (Bedford, 1995). Resistant starch of grain is also challenging for

chickens, and Brown (1996) reported that the RS3 is created during feed processing and storage. Resistant starch results from the processing of starch at a high temperature followed by storage at a low temperature over a long period of time. The starch in high moisture grain also anneals after heat processing. The normal structure of starch granules from maize (spherical, 10-16 microns across) with protein bodies and matrix creates a favourable environment for enzymatic digestion (Taylor and Belton, 2002).

Grains may be subjected to heating, for example during pelleting. The extent to which these processes occur during routine production and processing of maize has not been well documented. Furthermore, further drying of LM maize for a prolonged period of time may also cause resistant starch following stack-burn. Stack-burn is the deterioration in colour and quality of dried grains which have not been sufficiently cooled after drying/processing, then stored in stacks, which cool slowly. The quality of diets with stack-burned grains is low, tending to negatively affect broiler performance.

There is a need to assess the response of maize grain to high temperature treatment. In this experiment such response is examined in maize from different sources when heat treated at a constant temperature over different time periods.

3.2 MATERIALS AND METHODS

3.2.1 Maize samples

Three commercial maize samples were obtained from three different locations, namely Moree in NSW, and Emerald and Darling Downs in QLD, Australia. These had been sun-dried to a dry matter content of 87.7, 88.2 and 88.3 %, respectively for Moree, Emerald and Darling Downs.

3.2.2 Drying methods

Once received the maize were split into three batches for each source. One batch (sun-dried) was assessed without further drying. The other two batches in each source were dried artificially using a forced draught oven at 105 °C for 30 min or 24 h respectively. After the drying process, the warm samples were placed in paper bags and cooled at

room temperature overnight. Upon cooling, the samples were kept in sealed air-tight bags prior to grinding.

3.2.3 Laboratory analyses

3.2.3.1 Proximate analyses

For chemical analyses, representative grain samples were ground by hammer mill, passed through a 1 mm sieve and then stored in a sealed glass bottle at 4 °C until the analyses. All analyses of grain samples were carried out in duplicate and the results are expressed on dry matter (DM) basis. The proximate analyses were done according to the methods of the Association of Official Analytical Chemists (AOAC, 2002). These methods were applied to DM, crude protein (CP), ether extract (EE), gross energy (GE) and ash analyses. Relevant controls and standards were included in all analytical procedures, to confirm the reliability of the methods.

For DM analysis, samples were accurately weighed (2–4 g) in duplicates into pre-weighed silica crucibles and placed in a forced-air convection oven (Qnaltex Universal Series 2000, Watson Victor Ltd., Perth, Australia) which was preheated to 105 °C. Samples were maintained at this temperature overnight or until constant mass was obtained. The DM content is the ratio of the dry weight to the pre-dried weight expressed as a percentage.

The nitrogen content of grain samples was determined according to the DUMAS combustion technique following the method described by Sweeney (1989) using a LECO® FP-2000 automatic nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA). Nitrogen was freed by combustion at high temperature in pure oxygen and was measured by thermal conductivity detection and converted to equivalent CP by a numerical factor of 6.25. The furnace temperature was maintained at 105 °C for hydrolysis of sample in ultra high purity oxygen. To interpret the detector response as percentage nitrogen (w/w) calibration was done using pure primary standard of ethylenediaminetetra-acetic acid (EDTA).

The EE was determined indirectly by the Soxhlet Method for fat extraction. Around 2 g of finely ground sample was weighed into pre-weighed dry paper thimbles and extracted for 24 h with chloroform using a Soxhlet apparatus. Thimbles with samples were allowed to drain and dry at 105 °C for 24 h. The EE was calculated as percent of the loss of weight and expressed as a proportion of dried sample weight.

The GE contents of maize samples were determined using an IKA ® - WERKE bomb calorimeter (C7000, GMBH & CO., Staufen, GERMANY) at UNE. The GE value of maize samples was obtained as MJ/kg directly from the digital system of the calorimeter.

The ME (metabolizable energy) was calculated according to the equation:

ME (Kcal/kg) = [53 + 38 × (% CP + 2.25 × % EE + 1.1 × % Starch + % Sugar)], where CP is crude protein and EE is ether extract (Carpenter & Clegg, 1956). The values were converted to MJ/kg using a conversion factor of 1 MJ = 239 Kcal.

For crude ash determination, approximately 4 g dried ground maize grain samples were weighed into pre-weighed silica crucibles. The samples were ignited in a preheated Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK) at 600 °C for three hours. The Furnace temperature was set at 350 °C initially and then raised to the target temperature after one hour. The crude ash content was calculated using the following equation and expressed as:

$$\text{Ash \%} = \frac{\text{Ashed sample weight}}{\text{Dried sample weight}} \times 100$$

3.2.3.2 Phytate-P content

The following solutions were required for assaying phytate-P:

Solution 1 (Ferric solution), made by dissolving 0.2 g of ammonium iron (III) sulphate.$12H_2O$ (Merck kGaA) in 100 mL of 2N HCl and made up to 1000 mL with Milli-Q water.

Solution 2 (2,2-Bipyridine solution), prepared by dissolving 10 g of 2,2-bipyridine (Merck KGaA, 5427) and 10 mL thioglycollic acid (Merck Art, 700) in Milli-Q water and made up to 1000 mL.

The phytate-P content of grain samples was determined following a sensitive method for the rapid determination of phytate-P in cereals and cereal products as previously described (Haug and Lantzsch, 1983). Around 0.06 g of finely ground grain sample was weighed into 16 mL test tubes and 10 mL of 0.2M HCl solution was poured onto it. After that, 2 mL of Solution 1 was added and the tubes were heated in boiling water for 30 min allowed to cool down at room temperature. Then 2 mL of solution was added and the contents thoroughly mixed. Phytate-P content was determined colorimetrically at an absorbance of 519 nm by reading against a blank within one minute of adding bipyridine solution.

3.2.3.3 Starch content and composition

The starch and resistant starch contents of the maize samples were determined using the Megazyme total starch kit (AMG/AA 05/2006) based on the method developed by McCleary *et al.* (1994). This method is recognized by the AOAC Official Method 996.11 (AOAC 996.11) and the AACC Method (76.13) as well as the ICC (ICC Standards Method No. 168). Finely ground samples (0.5 mm) of approximately 100 mg were weighed accurately into screw-capped reaction tubes (30 mL) and wet with 0.2 mL 80 % ethanol. A further 3 mL of thermo stable α-amylase (3000 U/mL; 45 U/mg at pH 6.0, Megazyme) in MOPS buffer (50 mM, pH 7.0) were added followed by 0.1 mL amyloglucosidase (3300 U/mL on soluble starch at pH 4.5, Megazyme) and incubated at 50 °C for one hour. Glucose was determined colorimetrically after incubating an aliquot (0.1 mL) with 2.25 mL of GOPOD reagent (Megazyme) at 50 °C for 20 min and reading the absorbance at 510 nm against a reagent blank. For resistant starch determination, samples were treated with DMSO at 100 °C followed by enzymatic hydrolysis of starch as described above.

3.2.3.4 Amylose and amylopectin

The amylose/amylopectin ratio was determined with a Megazyme amylose/amylopectin assay kit (Megazyme International Ireland, Bray Business Park, Bray, Ireland) using the selective quantitative precipitation reaction of con-canavalin A (Con A) for amylopectin (Gibson *et al.*, 1996) and by the colorimetric method of iodine binding for amylose (Chrastil, 1987). Finely ground samples (20–25 mg) were weighed accurately into screw-capped reaction tubes (30 mL) and wet with 1 mL DMSO and put into a heating block at 100 °C for 20 min. After cooling, 2 mL of 95 % (v/v) ethanol was added. A further 4 mL of pure ethanol were added and left overnight for starch precipitation. After blowing dry (under N_2), 2 mL of DMSO were added and put into a 100 °C heating block for exactly 15 min after which 8 mL of Con A Solvent was added and the volume was mixed thoroughly (Solution A). Later on, 0.4 mL of Solution A was transferred to a 2 mL microfuge tube and 0.50 mL of Con A was added and gently mixed by repeated inversion. After one hour, the microfuge tubes were centrifuged at 14 000 × g for 10 min. This was followed by transferring 0.5 mL of the supernatant to a 16 mL centrifuge tube and adding 1.5 mL of sodium acetate buffer (100 mM, pH 4.5). After denaturing the Con A, 0.1 mL of amyloglucosidase was added and incubated at 40 °C for 40 min. Glucose was determined colorimetrically after incubating an aliquot (0.01 mL) with 2 mL of GOPOD reagent (Megazyme) at 40 °C for 20 min and reading the absorbance at 510 nm against a reagent blank.

3.2.3.5 Non-starch polysaccharides (NSP)

Non-starch polysaccharides were analyzed by gas chromatography (VARIAN, CP - 3800, USA) as the alditol acetate derivatives of monosaccharide. The insoluble residue after the extraction of samples with 80 % ethanol was dried to a slurry using nitrogen before incubation at 100 °C for 30 min to gelatinize the starch. Starch was removed enzymatically by incubation in 10 mL of 0.1 M acetate buffer pH 5.0 with 50 µL thermostable α-amylase (3000 U/mL; 45 U/mg, Megazyme) at 95 °C for 30 min and after cooling with amyloglucosidase (3300 U/mL on soluble starch at pH 4.5, Megazyme) at 55 °C for 16 h. After incubation, the samples were centrifuged at 2000 ×*g* for 30 min. Insoluble NSPs were determined by drying the insoluble residue under

nitrogen and hydrolyzing with 12 M H_2SO_4 for 1 h at 30 °C, followed by hydrolysis in 1 M H_2SO_4 for 2 h at 100 °C (Seaman *et al.*, 1963). The hydrolysate was cooled to room temperature and an aliquot of 0.8 mL was transferred into a clean 30 mL reaction tube and 0.2 mL of 28 % NH_3 was added. The sample was thoroughly mixed and precisely 50 µL of two internal standards (inositol 4 mg/mL and allose 4 mg/mL) were added.

The mixture was dried in a vacuum rotary evaporator (8 h at 40 °C). The residue was recovered in distilled water (0.2 mL) and monosaccharides were reduced by treatment with sodium borohydrate ($NaBH_4$) (0.3 mL, 50 mg sodium borohydrate per mL 3 M NH_4OH) at 40 °C for 1 h. The excess NaBH4 was decomposed with glacial acetic acid. The alditol acetate derivatives were acetylated by the addition of acetic anhydride (5 mL) in the presence of 90.5 mL of 1-methylimidazole. Excess acetic anhydride was decomposed with 5 mL of distilled water and the alditols were extracted with 2 mL of dichloromethane. The volatile alditol derivatives of monosaccharides were analysed using a Varian, CP-3800 gas chromatograph equipped with Varian series, auto-sampler, a capillary column and a flame ionization detector set at 280 °C. During analysis, the column was held at 195 °C for 1 min and then raised by 5°C/min to 225 °C and held for 4 min. Soluble NSPs were measured by taking an aliquot of the supernatant after starch removal and precipitating soluble NSP in 80 % ethanol by adding 16 mL of absolute ethanol to 4 mL of supernatant. The precipitate was dried under a stream of nitrogen at 40 °C, and 1 mL of 2 M trifluroacetic acid was added. The mixture was hydrolyzed at 125 °C for 1 h. After cooling, precisely 50 µL of two internal standards (inositol 4 mg/mL and allose 4 mg/mL) were added and the trifluroacetic acid was removed by co-distillation with distilled water under a stream of nitrogen. The dry residue was recovered in distilled water (0.2 mL) and the monosaccharides were reduced and acetylated as described earlier in this chapter. The levels of polysaccharides were calculated from the level of the component sugars using a polymerization factor, 0.88, for pentose (ribose, xylose and arabinose), 0.9 for hexoses (mannose, galactose and glucose), 0.89 for deoxysugars (fructose and ribose) and 0.91 for rhammnose as recommended by Englyst and Hudson (1993), and Theander and Westerlund (1993).

Free sugars (monosaccharide and oligosaccharides)

Free sugars were analyzed by gas chromatography (VARIAN, CP-3800, USA) as the alditol acetate derivatives of monosaccharides. Around 100–200 mg of finely ground maize samples were placed in screw-capped glass vials and 5 mL of hexane were added. The samples were vortexed, sonicated for 15 min and centrifuged to remove fat. The residue was extracted with a 5 mL ethanol:water mixture (80:20), at 80 °C for 10 min to remove free sugars and oligosaccharides. After centrifuging, the supernatant was collected. Hydrolysis, reduction and acetylation of the samples were carried out following the procedures described by Theander and Westerlund (1993). After drying the supernatant in a vacuum rotary evaporator (6 h at 40 °C), 3 mL of 1 M H_2SO_4 was added and the samples were hydrolyzed at 100 °C for 2 h. The hydrolysates were cooled to room temperature and an aliquot of 0.4 mL was transferred into a clean 30 mL reaction tube and 0.1 mL of 28 % NH_3 was added. The samples were thoroughly mixed and precisely 50 μL of two internal standards (inositol 4 mg/mL and allose 4 mg/mL) were added. The mixture was dried in a vacuum rotary evaporator (4 h at 40 °C). Reduction and acetylation were carried out using the same procedure as described for the determination of soluble NSP earlier in this chapter.

3.2.3.6 Amino acid composition

Concentrations of amino acids were determined using pre-column derivatisation amino acid analysis with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by separation of the derivatives and quantification by reversed phase high performance liquid chromatography (HPLC) according to Cohen and Michaud (1993), and Cohen (2001). Amino acids were detected by UV absorbance. Approximately 100 mg of sample were hydrolyzed in 20 % HCl for 24 h at 110 °C. An internal standard (α amino butyric acid; AABA) was added to each sample following hydrolysis. Ten μL of the solution were then derivatised using an AccQ Tag Ultra Derivatization Kit (Waters Corp. USA; 70 μL borate buffer + 20 μL AccQ Tag solution, incubated 10 min at 50 °C). The HPLC analysis was based on the method of Cohen (2001) but adapted for use with an ACQUITY Ultra Performance LC (UPLC; Waters Corp. State USA) system. The column employed was an ACQUITY UPLC BEH C18 1.7 μm column (water) with

detection at 260 nm and a flow rate of 0.7 mL/min. Samples were analyzed in duplicates and results were expressed as an average. All of the above procedure was carried out by the Australian Proteome Analysis Facility (APAF), Macquarie University, NSW, Australia.

3.2.3.7 Isolation of soluble NSP

Ground maize samples were washed twice with 200 mL n-hexane. After air-drying the samples, starch and protein were enzymatically removed by incubation with 200 mL distilled water in a water bath (100 °C, 30 min) in the presence of a heat stable α-amylase (Termamyl 120 L, Type L, Novozymes A/S, Denmark; 0.25 mL, 95 °C, 30 min) followed by incubation with 0.5 mL amyloglucosidase (AMG, 300 L, Brewq, Novozymes A/S, Denmark) at 55 °C for 16 h. The mixture was cooled and centrifuged at high speed (7500 ×*g* for 30 min). The clear supernatant was then incubated overnight followed by adjustment with two volumes of ethanol to 66 % (v/v) and the precipitate was collected, blow-dried under nitrogen and then kept in a cool room at 5 °C until NMR analysis.

3.2.3.8 Nuclear Magnetic Resonance (NMR) analysis

The isolated NSP (10 mg) from maize grains was dissolved in 2 mL D_2O (99.9 atom % D) and freeze-dried. This step was repeated three times to remove interference by exchangeable protons. All spectra were acquired on a Bruker Avance 300NMR spectrometer using a 5 mm inverse 1H BB porbhead with a Z-gradient. 1H-NMR spectra were acquired at 88 °C in D_2O (100 atom % D) solution at a concentration of 20 mg/mL. Chemical shifts are expressed relative to an external acetone standard (2.2 ppm).

3.2.3.9 Mineral analysis

Minerals were analyzed by inductively coupled plasma (ICP) method (Vista MPX-radial) following a published protocol (Anderson and Henderson, 1986). The sealed chamber digest (SCD) method was also used for P, S, K, Na, Mg, and trace elements. This digest is the most appropriate for ICP analyses in which final oxidation occurs in

the high temperature plasma of the ICP. Ground maize samples, approximately 0.5 g, were weighed into 50 mL borosilicate reagent bottles and exact weights and vial numbers were entered on a record sheet. After that, 2 mL of a 7:3 (v/v) mixture of $HClO_4$ (70 %) and H_2O_2 (30 %) was added to each tube and were capped tightly. These were left overnight at room temperature for digestion before 1 mL of H_2O_2 was added, and the bottles tightly sealed and placed into a warming oven at 80 °C for 30 min. After cooling slightly, an additional 1 mL of H_2O_2 was added and they were left for one hour longer for further digestion. The final volume was adjusted to 25 mL of total volume using distilled water and mixed thoroughly. Before reading the absorption at 785 nm against a blank, the samples were briefly stored at 2 °C to reduce absorption onto the plastic and inhibit the growth of microorganisms.

3.2.3.10 *In vitro* digestibility

The *in vitro* digestibility of dry matter, starch and crude protein was determined by the method of Babinszky (1990) with slight modifications. A volume of 12.5 mL of 0.1 N HCl containing 4 g/L pepsin (Sigma Chemical, St Louis. Mo, USA) and the tested enzyme were mixed with 500 mg of maize samples and then the mixture was gently shaken in a water bath at 40 °C for 1.5 h. After neutralization with 110 mg $NaHCO_3$ (2 mL in each tube), the digesta was mixed with 12.5 mL of 0.2 M potassium phosphate buffer containing pancreatin (4 g/L) and amylase (4 mL/L) and incubated to stimulate the pancreatic phase at 40 °C for 3 h with occasional vortexing. After the incubation period, 2.5 mL of $NaCO_3$ (100 g/L) was added to each tube and the contents were centrifuged at 1643 × *g* for 20 min. Afterwards, the supernatant was kept on ice until the viscosity analysis and the residue was repeatedly rinsed with MQ water. The residue was used for the calculation of DM and determination of glucose, which was converted to starch for starch digestibility. The digestibility of DM, starch and crude protein (CP) was calculated by the following equations:

$$\text{DM digestibility (\%)} = \frac{\text{Weight of maize sample} - \text{Weight of dried residue}}{\text{Weight of maize sample}} \times 100$$

$$\text{Starch digestibility (\%)} = \frac{\text{Starch\% of maize sample} - \text{Starch\% of dried residue}}{\text{Starch \% of maize sample}} \times 100$$

$$\text{CP digestibility (\%)} = \frac{\text{CP\% of maize sample} - \text{CP\% of dried residue}}{\text{CP\% of maize sample}} \times 100$$

***In vitro* viscosity**

The *in vitro* viscosity (in centipoises, cP = 1/100 dyne second per cm^2) of grain samples was measured on the thawed supernatants with a Brookfield DVIII viscometer at 25 °C with a cP 40 spindle at 100 rpm according to the method described by Bedford and Classen (1993). The samples did not exhibit shear thinning at these shear rates. The samples were reconstituted and finely ground before doing the *in vitro* viscosity.

3.2.3.11 Electron microscopy

The whole maize grain was scoured around the edges, then frozen in liquid nitrogen and fractured. They were then mounted on 12 mm aluminum stubs using double-sided adhesive tape and gold-coated using a Polaron E 5100 sputter coater. The structure of the starches of the samples was observed using an electron microscope (JSM-5800LV scanning microscope JEOL, 1993). All micrographs were taken at a magnification of × 500.

3.2.4 Statistical analysis

The data were subjected to sample one test using SPSS Statistics version 17.0.0 (SPSS Inc, 2009) for average and standard error followed by calculation of the coefficient of variation (CV).

3.3 RESULTS

3.3.1 The morphological structural changes of maize grains

The images of starch granules of maize samples with different sources found by scanning under electron electronic microscope are shown in Plate 3.1. The starch granules of the samples from different sources appeared morphologically similar to one another in the case of sun-dried grains, while granules of samples from Moree were loosely packed with a normal spherical shape and a lot of protein matrix. After 30 min of heating, the shapes of starch granules changed and all sources of grains became shrunken and distorted. The surfaces of the starch granules were rougher, irregular and elongated. Following 24 h of heating the granules were found to be closely packed, irregular, and rough in shape compared to the sun-dried samples. In Emerald, the starch granules were bigger and more flattened than the sundried granules and this was observed after 30 min heating, whereas after 24 h heating, they were more tightly packed with protein matrix.

The electron microscope images for the Downs maize revealed that the starch granules were squeezed and deformed and had more protein matrix than the sun-dried samples. After 24 h of heat treatment, the starch granules were more constricted and were overlapped each other. Another characteristic feature was that the granules were tightly packed in contrast to the sun-dried maize. Most protein bodies are located outside the starch granules and they were enveloped by the starch granules as well. This was observed in all the maize sources after 30 min of heating.

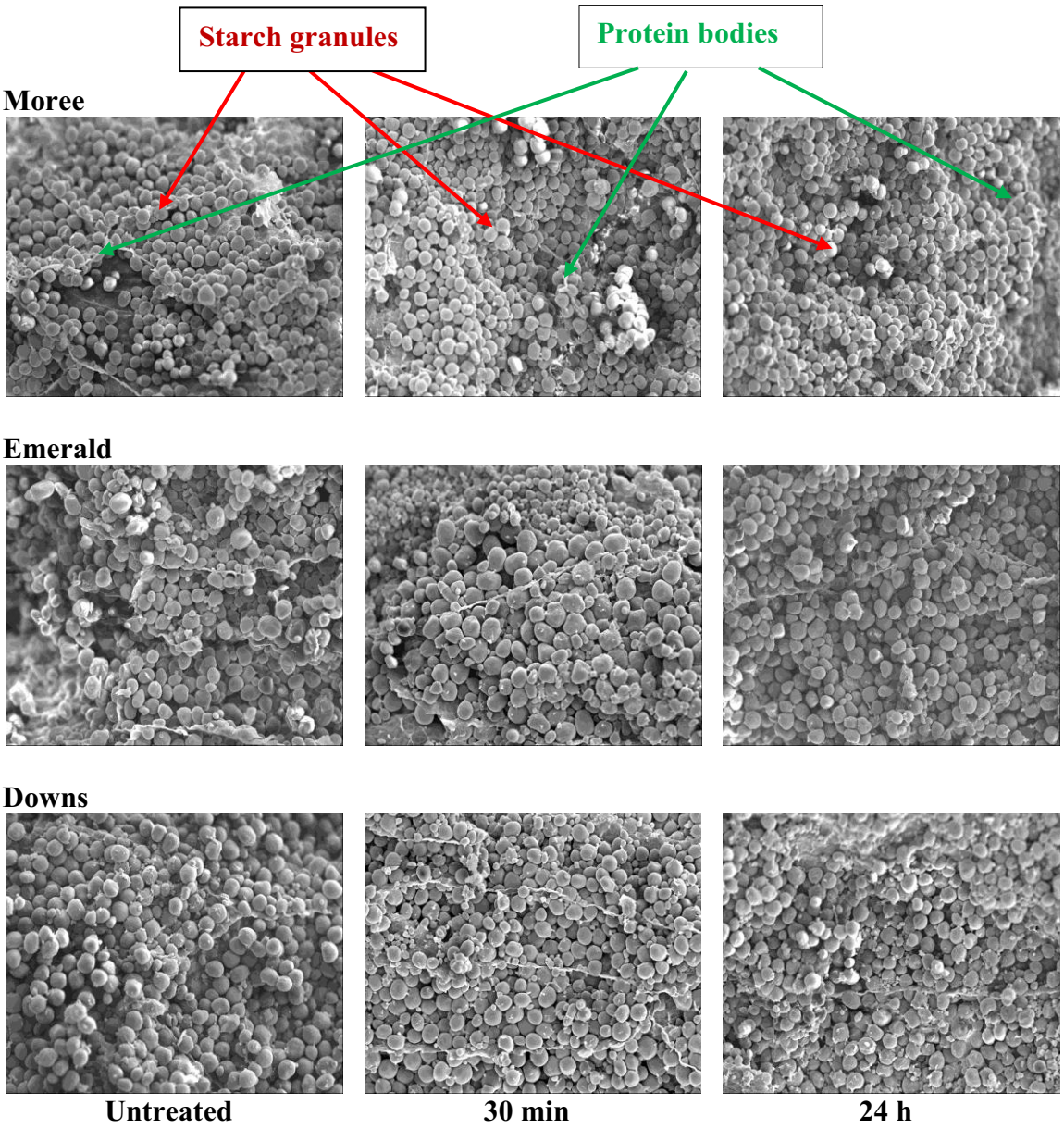


Plate 3.1 Electron micrographs (× 500) of Moree (top panel), Emerald (middle panel) and Downs (bottom panel) maize as untreated or artificially heat treated at 105 °C for varying durations

3.3.2 Proximate composition

The proximate composition of different sources of maize with or without heat treatment is shown in Table 3.1. Mean values of DM and GE were increased and varied between 877.0 and 960.2 g/kg and 18.6 and 18.9 g/kg respectively with an increase in drying period for Moree. However, EE, phytate-P and ME were decreased from 65.9 to 44.9

g/kg, 2.5 to 1.9 g/kg and 16.5 to 16.0 MJ/kg, respectively under the same conditions. There was no clear trend in changes in the CP and ash contents as samples were heated.

The DM content of the Emerald samples varied between 882.8 and 963.9 g/kg, increasing as samples were heat-treated. The CP, EE, and phytate-P decreased and varied between 93.1 and 92.7 g/kg, 67.1 and 49.6 g/kg and 2.2 and 1.5 g/kg, respectively under the same conditions. Heat treatment had no effect on ash, GE and ME contents of the Emerald maize.

In the Downs maize DM, CP, EE, phytate-P and ash content but not GE and ME were affected by heat treatment. The DM, CP and ash contents increased and varied between 883.4 and 967.4 g/kg, 101.2 and 102.1 g/kg and, 14.0 and 14.8 g/kg, respectively with an increase in drying period at 105°C. In terms of CV values, EE, phytate-P and ash were found to be the most variable components in the maize grains.

3.3.3 Starch content and composition

The effects of heat treatment on starch content and composition in different sources of maize are presented in Figure 3.1. In the Moree sample, the total starch content slightly increased with an increase in heating duration at 105 °C compared to sun drying sample, while the total starch content decreased for the Emerald and Downs maize. However, in all three sources of maize the total starch content did not change considerably after 30 min of heating. The Emerald maize was found better in terms of total starch content as compared with others.

The amount of digestible starch was slightly decreased by heat treatment of maize grains at 105 °C over different durations irrespective of the source of maize. The trend from 30 min to 24 h of heating at 105 °C was more or less the same in all sources of maize. The resistant starch content increased sharply with increase in heating period at 105 °C for all sources of maize. The trend was similar in the case of Emerald and Downs maize in contrast to Moree maize.

Table 3.1 Proximate composition (g/kg DM) of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

Treatments								
Sources	**Heating period**	**DM[1]**	**CP[1]**	**EE[1]**	**PhytateP[1]**	**Ash[1]**	**GE[2]**	**ME[2]**
Moree	Untreated	877.0	108.3	65.9	2.5	19.4	18.6	16.5
	30 min	897.6	107.2	49.0	2.4	15.8	18.7	16.0
	24 h	960.2	108.7	44.9	1.9	19.7	18.9	16.3
Emerald	Untreated	882.8	93.1	67.1	2.2	15.2	18.8	16.4
	30 min	898.7	92.1	53.2	1.9	14.7	18.7	16.1
	24 h	963.9	92.7	49.6	1.5	15.5	18.8	16.4
Downs	Untreated	883.4	101.2	59.4	1.8	14.0	18.9	16.2
	30 min	902.3	101.5	57.5	1.7	14.1	18.9	16.3
	24 h	967.4	102.1	52.0	1.2	14.8	18.9	16.3
CV		*0.04*	*0.07*	*0.14*	*0.22*	*0.14*	*0.01*	*0.01*

[1] g/kg DM; [2] MJ/kg DM; CV = Coefficient of variation.

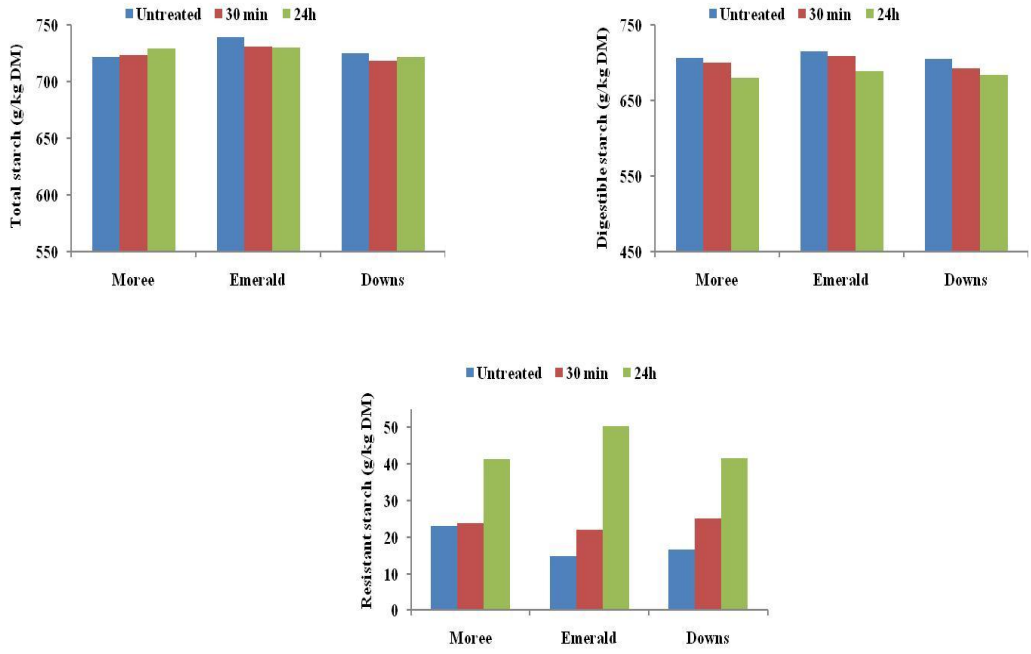


Figure 3.1 Total starches, digestible starch and resistant starch content (g/kg DM) of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

3.3.4 Amylose and amylopectin contents

The amylose and amylopectin contents of the maize batches are presented in Figure 3.2. The amylose content increased with increasing heating duration, the highest amylose content being in the Moree samples heated over 24 h. The same level of amylose content was found in untreated samples for all sources of maize. Dissimilar results were observed for amylopectin contents. The amylopectin content decreased with increased heating period at 105 °C for all sources of maize. The maize from Emerald and Downs had the highest amylopectin value of three sources.

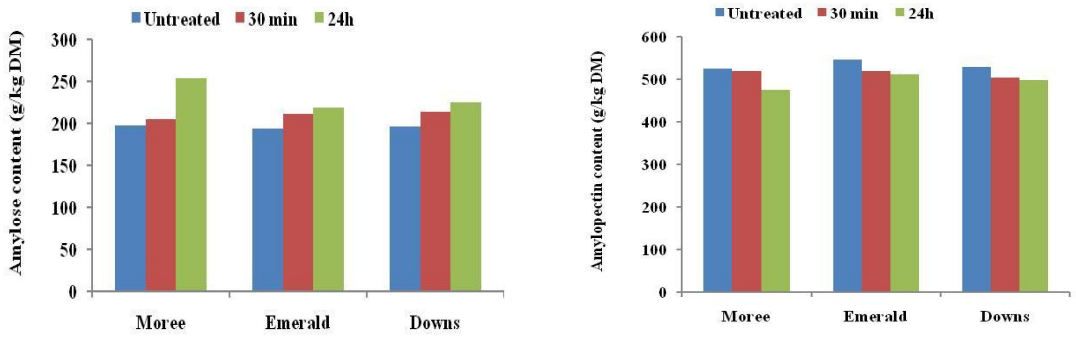


Figure 3.2 Amylose and amylopectin contents (g/kg DM) of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

3.3.5 NSP and free sugar contents

The results for NSPs are showed in Figure 3.3. The total NSP content decreased after 30 min of heating, but increased with 24 h of heating. The maize from Emerald and Downs had the highest total NSP content of the three. Moree and Emerald maize had a similar soluble NSP content, but the trend shown by Downs was different to the other two sources.

In the Downs maize, the soluble NSP content increased with increase in heating duration. Moree and Emerald were the best sources of the three in terms of soluble NSP content. The levels of insoluble NSP increased with increase in duration of heating at the same temperature for all sources. Downs maize had the highest insoluble NSP content.

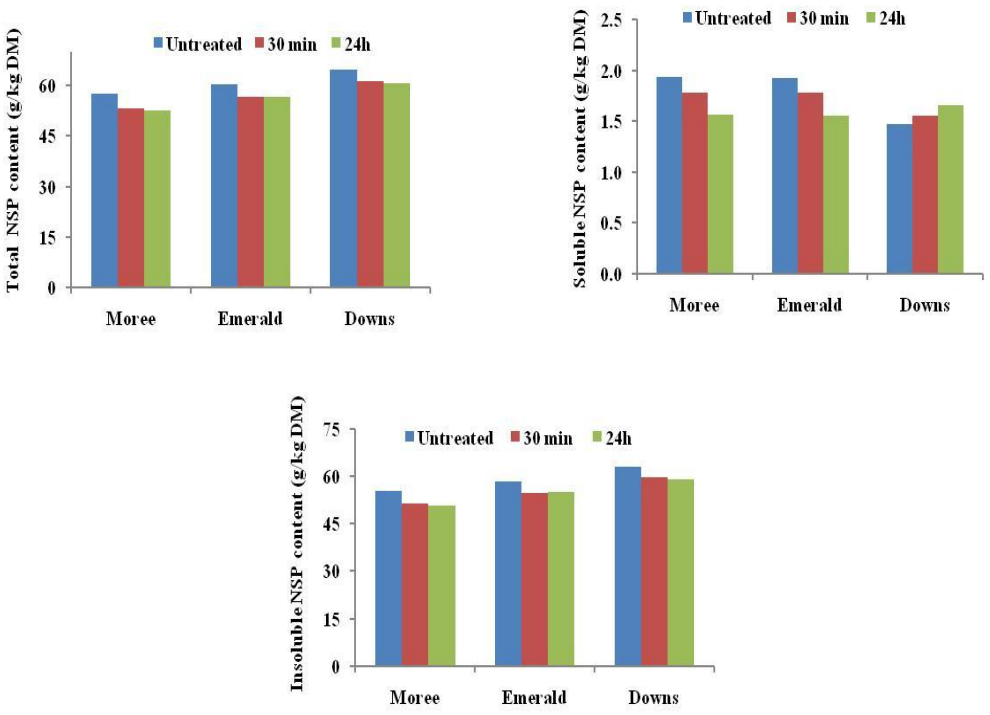


Figure 3.3 Concentration of non-starch polysaccharides (g/kg DM) of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

Available free sugar contents were determined for all batches of maize (Table 3.2). For maize from Moree the arabinose, xylose, mannose and galactose contents decreased with an increase in heating period while the glucose content increased. In the case of Emerald and Downs, there was little effect on arabinose, xylose and mannose contents due to heating when compared to the untreated maize. However, galactose and glucose contents decreased with the increase in oven drying duration at the same temperature. Xylose, galactose, arabinose and mannose were the most variable components of these free sugars in terms of CV.

3.3.6 Amino acid contents

The amino acid composition of the maize batches is shown in Table 3.3. They were consistently different with sources. The available amino acid content increased with an increase in the oven drying period in all maize samples. In addition, the amino acid contents increased considerably after 24 h oven drying period compared to the untreated

samples. Moree and Down had the highest histidine, threonine, alanine, lysine, methionine, isoleusine, leusine and phenylalanine. The Emerald maize had the lowest amino acid contents of the three sources.

Table 3.2 Available free sugar contents (g/kg DM) of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

Treatments							
Sources	**Heating period**	**Arabinose**	**Xylose**	**Mannose**	**Galactose**	**Glucose**	**Total**
Moree	Untreated	0.5	0.5	1.1	1.9	12.5	16.5
	30 min	0.3	0.3	1.0	0.7	12.6	14.9
	24 h	0.3	0.2	0.8	0.6	12.8	14.8
Emerald	Untreated	0.3	0.1	1.1	0.9	14.7	17.2
	30 min	0.2	0.1	1.0	0.8	14.2	16.4
	24 h	0.3	0.9	1.0	0.7	13.5	16.5
Downs	Untreated	0.3	0.2	1.0	1.0	14.7	17.3
	30 min	0.3	0.1	1.0	0.9	13.6	16.0
	24 h	0.3	0.1	0.8	0.8	13.9	15.9
CV		*0.18*	*0.94*	*0.11*	*0.43*	*0.06*	*0.05*

CV = Coefficient of variation.

Table 3.3 Amino acid contents (g/kg DM) of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

Treatments									
Sources	**Heating period**	**His**	**Thr**	**Ala**	**Lys**	**Met**	**Ile**	**Leu**	**Pal**
Moree	Untreated	2.6	3.2	7.1	2.5	1.3	3.4	12.1	4.7
	30 min	2.8	3.3	7.4	2.8	1.5	3.5	12.3	4.9
	24 h	2.9	3.6	8.1	2.8	1.7	3.9	13.8	5.4
Emerald	Untreated	2.2	2.8	6.2	2.3	1.2	2.9	10.2	4.1
	30 min	2.3	2.9	6.5	2.4	1.2	3.1	10.9	4.3
	24 h	2.5	3.0	6.8	2.6	1.3	3.3	11.2	4.5
Downs	Untreated	2.6	3.2	6.8	2.8	1.4	3.3	11.2	4.7
	30 min	2.7	3.3	7.0	2.9	1.4	3.4	11.6	4.9
	24 h	2.8	3.5	7.4	3.0	1.6	3.6	12.0	5.0
CV		*0.09*	*0.08*	*0.08*	*0.09*	*0.12*	*0.08*	*0.09*	*0.08*

CV = Coefficient of variation.

3.3.7 Nuclear magnetic resonance (H1 NMR) shifts

The anomeric regions of the H1 NMR of the three isolates from each source of maize are shown in Figure 3.4. A variable anomeric peak was observed for each and every treatment and the protons from the neutral sugars were resonating (4.6 to 5.2 ppm). In Moree maize, the anomeric proton peak of, for instance, α-glucose, isomaltose and β-

glucose increased gradually with the duration of oven drying at 105 °C compared with the untreated sample. An unknown peak, next to the β-glucose was observed only with the untreated Moree sample. In Emerald maize, very consistent peaks of anomeric protons were observed, increasing gradually with the increase in heating duration of samples. However, an unknown peak was also found just next to the β-glucose peaks with the 30 min and 24 h samples of Emerald grains. Dissimilar anomeric proton peaks were observed in the maize obtained from Downs. For example, the highest peaks of those protons were found in the grain samples heated for 30 min.

3.3.8 Mineral contents

In the Moree maize, Ca, P, Cu, Mn, Zn and Na contents decreased with heating (Table 3.4). The variation between the untreated and heated samples was 0.08 and 0.14 g/kg, 3.4 and 3.7 g/kg, 1.4 and 2.1 mg/kg, 7.6 and 8.8 mg/kg, 16.1 and 17.7 mg/kg and 73.0 and 119.5 g/kg, respectively. On the other hand, heat treatment had no effect on Mg content. A lower Fe value (20.1 mg/kg) was observed in Moree sample after heating at 105 °C for 30 min compared to untreated samples (35.5 mg/kg); however, there was a moderate increase (30.8 mg/kg) after 24 h heating at the same temperature.

In the case of Emerald sample, the values for Ca, Mg and P showed little change after 30 min heating, however, the content of Ca and P dropped and Mg remained the same after 24 h heating in compared to the untreated. The Fe, Cu, Mn and Zn values increased with increase in heating period. In contrast, the content of Na decreased with heating compared to untreated Emerald sample.

Downs sample had different results from the other two sources. The values of Ca, Mg, P, Fe, Cu, Mn and Zn increased with increase in heating time compared to the control. On the other hand, the Na value decreased. The values of Mg, P and Fe increased and other minerals contents were reduced after 24 h of heating when compared to untreated maize.

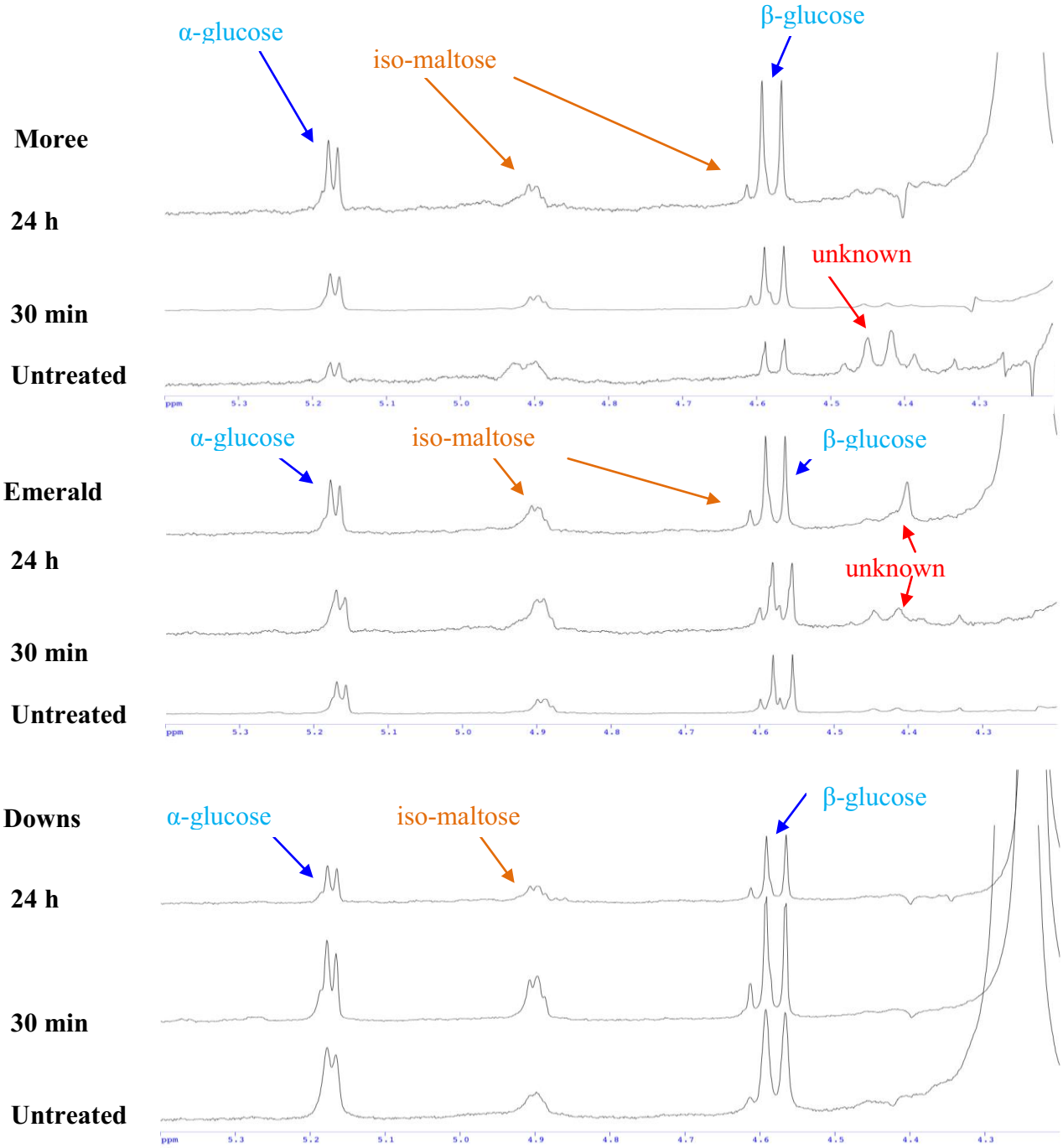


Figure 3.4 Region of H1 NMR spectrum showing anomeric protons of the three maize samples with untreated or artificially heat treated at 105 °C for varying durations

Table 3.4 Concentration of macro and trace minerals of maize of different sources as untreated or artificially heat treated at 105 °C for varying durations

Treatments									
Sources	**Heating period**	**Ca** g/kg	**Mg** g/kg	**P** g/kg	**Fe** mg/kg	**Cu** mg/kg	**Mn** mg/kg	**Zn** mg/kg	**Na** mg/kg
Moree	Untreated	0.14	1.4	3.6	35.5	2.1	8.8	17.7	119.5
	30 min	0.09	1.5	3.7	20.1	1.5	8.2	16.1	74.0
	24 h	0.08	1.4	3.4	30.8	1.4	7.6	16.2	73.0
Emerald	Untreated	0.11	1.3	2.9	13.0	1.2	6.1	12.7	110.6
	30 min	0.11	1.2	2.9	12.7	1.7	5.9	11.9	82.2
	24 h	0.05	1.2	3.1	12.3	1.4	5.9	11.6	71.9
Downs	Untreated	0.08	0.9	2.5	16.9	2.3	7.0	20.3	81.2
	30 min	0.09	1.1	2.9	20.1	2.3	7.8	22.4	78.7
	24 h	0.08	1.1	2.7	25.0	2.9	7.8	23.5	72.1
CV		*0.29*	*0.15*	*0.14*	*0.39*	*0.30*	*0.15*	*0.26*	*0.21*

CV = Coefficient of variation.

3.3.9 *In vitro* digestibility

The mean % *in-vitro* DM, starch and protein digestibility of Moree batches varied between 49.5 and 53.8, 56.7 and 58.8, and 43.3 and 46.1, respectively (Table 3.5). The digestibility of DM, protein and starch was improved by heat treatment of the samples. A similar pattern was observed in *in vitro* DM and starch digestibility of Emerald maize batches except protein digestibility and these values varied between 49.4 and 52.1 %, 55.1 and 57.4 %, respectively. *In vitro* protein digestibility was not varied due to heat treatment of Emerald maize. Analogous trends were observed for these variables in the Downs's maize at the same drying temperature, but the range was narrower than in the other two sources of maize. For example, DM digestibility varied between 52.8 and 53.8 %, starch digestibility between 53.6 and 55.2 %, protein digestibility between 44.8 and 48.9 %. *In-vitro* protein and starch digestibility were the most variable in terms of the CV.

3.3.10 *In vitro* viscosity

The *in vitro* viscosity decreased and varied between 0.90 and 0.93 cP for Moree batches (Table 3.5). A similar pattern was observed in Emerald samples and the value varied between 0.90 and 0.94 cP. Analogous trends were observed for this variable in the

Downs's maize at the same drying temperature, but the range was narrower than in the other two sources of maize such as the value varied between 0.89 and 0.91 cP.

Table 3.5 *In vitro* dry matter, starch and CP digestibility and viscosity of maize batches as untreated or artificially heat treated at 105 °C for varying durations

Treatments		Digestibility (%)			Viscosity[1]
Sources	**Heating period**	**DM**	**Starch**	**Protein**	
Moree	Untreated	49.5	56.7	43.4	0.93
	30 min	52.1	57.1	47.4	0.92
	24 h	53.8	58.8	46.1	0.90
Emerald	Untreated	49.4	55.1	55.9	0.94
	30 min	50.6	56.4	56.2	0.91
	24 h	52.1	57.4	55.2	0.90
Downs	Untreated	52.8	53.6	44.8	0.91
	30 min	53.2	54.1	52.9	0.89
	24 h	53.8	55.2	58.9	0.89
CV		*0.03*	*0.06*	*0.11*	*0.02*

[1] cP (in centipoises) = 1/100dyne second per centimetre squared; CV= Coefficient of variation.

3.4 DISCUSSION

3.4.1 Morphological structure of maize grains

It is clear from the current study that maize starch granules, protein bodies and matrix were affected by artificial heating compared to untreated sample. With respect to starch, different types of starch granules were observed in different maize sources after heat treatment. The starch granules of maize are large, essentially spherical granules 2–3 μm in diameter (Taylor and Belton, 2002; Tester *et al.*, 2004), and a similar structure was observed in this study in the case of the untreated maize grain. On the other hand, most of the starch granules were found to be more compressed and some were overlapping each other, and they were tightly packed, after heat treatment. It is speculated that differences in size and shape of starch granules within each variety as well as between sources was possibly due to less moisture and overheating. However, the size of the granules does alter after artificial drying of grains. The size of the starch granule is an important factor in determining the energy value of starch, with smaller granules having a relatively larger surface area and so a greater potential for hydrolysis by endogenous amylase (Carre, 2004). During heat processing, the starch gelatinizes to an extent that is

dependent on granule size, moisture content, amylose: amylopectin ratio and storage time (Klucinec and Thompson, 1999; Tester *et al.*, 2004).

It is speculated that these changes may simply be a consequence of the maize kernel being borne on the top of the plant and exposed to direct sun and higher temperatures, rather than being in the shade and in slightly cooler temperatures. Starch granules are embedded to varying degrees in protein matrix and this protein must be degraded to expose the starch fully to amylases. The protein matrix surrounding the starch granules in maize contains kafirins with many disulphide bonds which are particularly resistant to enzyme attack (Rooney and Pflugfelder, 1986). It was postulated by Peplinski *et al.* (1994) that most changes in the physical properties of kernels became obvious only in maize dried at above 40 °C.

3.4.2 Proximate composition

Additional drying of maize grains with relatively low moisture contents resulted in a sharp decline in moisture content and a simultaneous increase in the concentrations of the solid components especially proteins and ash. Concentration of nutrients such as EE tended to decrease but GE and ME were minor increases as a consequence of the heating treatment. Similar results were found by Debora *et al.* (2004) who mentioned that chemical composition and energy (AME and AMEn) were positively influenced by drying temperature in maize-based diet. In addition, MacMasters *et al.* (1959) suggested that the alteration of composition and the physical state of kernels was due to artificial drying and season of harvest. However, Peplinski *et al.* (1994) showed only minor change in chemical composition of maize dried from 25 °C to 100 °C. Dissimilar results were found by Velu *et al.* (2006) who showed that there was no change in protein and starch contents due to drying. This may be due to use of lower temperature during the process.

Reduced levels of CP, EE and phytate-P in particular, in the Downs maize are an indication of increase in the DM content of grains with increase in drying temperature. These findings agree with those of Weller *et al.* (1988) who reported a reduction in protein contents as maize drying temperature increased, especially at high harvest moisture contents (>28 %). On the other hand, these disagree with the findings of earlier

researchers, who mentioned that these variables increased after artificial drying (Iji *et al.*, 2003). However, CP content increased in maize grain from other two sources due to artificial heating. This variation in CP content may be due to different sources of grain.

In this study, oven drying also resulted in an increase in the amino acid contents of the maize grains and these findings agree with those of Iji *et al.* (2003). The benefit for high temperature drying of maize is severe stress cracking resulting in increased accessibility of the protein matrix to enzymes (Eckhoff and Tso, 1991). It may also result in an increase in the concentrations of most essential amino acids. This may have some implication for feed formulation as grains tend to be classified with the same nutrient compositions regardless of how they were dried.

3.4.3 Starch composition

Discernible variations were found regarding starch contents due to heating for different durations as well as for different sources of maize, in this study. A higher drying temperature over an extended duration tended to increase the starch and amylose content of samples. Although resistant starch is formed during seed development, its proportion may increase during feed processing, especially drying (Brown, 1996) and storage. While most maize is sun-dried *in situ*, wet weather may necessitate the use of artificial drying techniques. Artificial drying results in the annealing of starch, while long periods of sun-drying have been known to cause stack-burn, a defect in a downgrading of the quality of the crop. The results differ from those of Monica and Costantino (1997) and Singh *et al*. (1998), who observed a decrease in starch yield of between 5.8 and 8.2 % when maize hybrids were harvested at high moisture contents and dried at 110 °C. Singh *et al.* (1998) found significant differences between maize hybrids in their sensitivity to drying in the sun or at 110 °C and in the resulting decrease in starch yield which varied from 7.2 to 15.1 % on the basis of hybrids. The researchers suggested that the effect of drying was not as brutal if the hybrids had been harvested at low moisture content.

Brown *et al.* (1981) reported that high-temperature drying lowers the yield and quality of starch and also starch yield decreases significantly as the drying temperature increases beyond 90 °C (Watson and Sanders, 1961). Generally, temperatures above the

gelatinization temperature of starch (approximately 57 °C) have a negative effect on starch yield and quality.

One of the theories as to why high-temperature drying decreases starch yield involves the thermal denaturation of proteases in the endosperm (Kerpisci, 1988). The current data support this theory and starch yield may be improved through protease supplementation.

In this study, amylose content increased and conversely amylopectin content decreased with an increase in the heating period. It needs to be stated that the stated effects on amylose and amylopectin values may stem from the differences in the three sources as well as from the artificial heating of maize grains. Once the thermal process is completed and the grain cools, retrogradation begins (Atwell *et al.*, 1988) and then starch returns to a more ordered state, where both amylose and amylopectin form double helical associations, the extent of which is dependent on the amylose: amylopectin ratio (Jacobson *et al.*, 1997; Klucinec and Thompson, 1999). Retrogradation is a reaction that takes place in gelatinized starch when the amylose and amylopectin chains realign themselves, causing the liquid to gel. When native starch is heated and dissolved in water, the crystalline structure of amylose and amylopectin molecules are lost and they hydrate to form a viscous solution. If the viscous solution is cooled or left at lower temperature for long enough period, the linear molecules of starch amylose, and linear parts of amylopectin molecules retrograde and rearrange themselves again to a more crystalline structure. The linear chains place themselves parallel and form hydrogen bridges. These changes in structure associated with heating also alter the subsequent digestibility of the starch.

It was noted that resistant starch content increased with an increase in the heating duration and this observation does support those of Berry (1986), Russell *et al.* (1989) and Iji *et al.* (2003) who observed that an increased RS content due to heat treatment of maize grain at 100 °C under an insufficient moisture condition. It has been reported that RS content increased from 20 to 65 % and physiochemical properties changed due to heat treatment (Itoh *et al.*, 1997). The decline in the content of amylopectin suggests deterioration in grain quality since amylopectin is more quickly digested than amylose and affects the digestion of nutrients other than carbohydrates.

3.4.4 NSP concentrations

In the current study, both soluble and insoluble NSP content increased as a result of heat treatment of maize grain. Soluble NSP is responsible for the reduction in value of diets based on temperate cereals but these are generally low in maize and other tropical cereals (Choct and Annison, 1992). This may be responsible for the low viscosity of the *in vitro* digesta in this research. The differing results observed could be due to the difference in the sources or the differing time periods of the oven drying.

3.4.5 Nuclear magnetic resonance (H1 NMR)

In the present study, it is clear that the nuclear magnetic resonance shifts were altered by artificial drying of low moisture maize grains over varying time periods. The changes in structure of the samples through heat treatment were further confirmed by H1-NMR analysis. Assignment of the major peaks were made by reference to data from the literature (Annison *et al.*, 1992; Cheetham *et al.*, 1993). The poorly resolved resonance of both NMR spectra makes interpretation difficult. Each H1-NMR peak increased sharply with an increase in the heating period of the grains. This is probably due to the higher content of glucose and maltose sugars in artificially dried maize.

3.4.6 Mineral contents

In the current study, it is obvious that the mineral contents of maize varied from one source to other and were changed through artificial drying of low moisture maize grains over varying time periods. This is a relative rather than absolute change, resulting from increase in DM with increase in drying time and temperature. For example, Fe and P contents were the most affected in the Moree samples while Cu and Zn were most altered in Emerald. The Ca and Mg contents of the Downs samples were most altered. Sodium content was greatly altered in all 3 samples.

3.4.7 *In vitro* digestibility

In the present study, the *in vitro* digestion of DM, starch and protein increased considerably with an increase in the duration of heating at 105 °C. This may be due to

the decreased amount of RS, as well as positive changes in the protein configuration of samples. These findings are supported by those of Nir *et al.* (1993) who reported a 4.5 fold increase in *in vitro* digestion of maize starch by amylase after heat treatment. The decrease in the content of resistant starch suggests an increase in grain quality, since resistant starch is unresponsive to animal enzymes (Noy and Sklan, 1994).

3.5 CONCLUSIONS

It can be concluded that the chemical composition, ultra-structural characteristics and energy values of maize samples varied from one source to the other and were changed through heating of low-moisture maize grains over varying time periods. In particular, there was increase in the concentration of protein, amino acids, total starch, resistant starch and total NSP due to loss of moisture with heating. Improvement in *in vitro* nutrient digestibility was also observed in artificially dried maize in contrast to untreated maize. Conversely there was a reduction in the concentrations of phytate-P, digestible starch, amylopectin and soluble NSP.

There was a change in ultra-structure of grain such as shape of starch granules, protein matrix of samples due to heat treatments. These changes could impact on the nutritive value of the grain and animal performance if diet formulations are only based on mean table values. For these reasons, it is recommended in diet formulation for broiler chickens to take into consideration the maize grain source, nature of drying and particularly its chemical composition. The impact of these changes on animal response warrants further investigations.

CHAPTER 4 EFFECTS OF GRAIN SOURCE, MILLING TECHNIQUE AND PARTICLE SIZE OF MAIZE ON FEED UTILIZATION, GUT PHYSIOLOGY AND GROWTH OF BROILER CHICKENS

4.1 INTRODUCTION

The nutritive value of grain largely depends on variety, season, source and harvest conditions. In addition to the quality, the utilization of energy from maize by broiler chickens depends on the preparation and processing of feed, particularly the particle size and milling technique (Reece *et al.*, 1985; Hamilton and Proudfoot, 1995; Kilburn and Edwards, 2001; Peron *et al.*, 2005; Parsons *et al.*, 2006). Coarse grain particle has been found to induce the development of gastro-intestinal organs particularly gizzard in young chicks, which ultimately contributes to productivity (Nir *et al.*, 1994b).

The season, location and variety of grains can have large effects on starch, soluble and insoluble NSP, protein, and AME of wheat, triticale and rye (Nyirenda *et al.*, 1987; Metayer *et al.*, 1993; Choct *et al.*, 1999a; Cowieson, 2005). Other cereals, for example, maize, sorghum, barley and oats were also relatively inconsistent across season and sites. The climatic conditions can also be responsible for the variations of starch content and granular structure, composition and hardness of grains (Tester *et al.*, 1995; Tester, 1997).

The percentage of fine particles obtained after grinding depends on the hardness of the grain with a higher percentage of fine particles from softer grains (Carre *et al.*, 2005). A harder endosperm gives larger particles with more irregular shapes, while a soft endosperm produces smaller sized particles (Rose *et al.*, 2001). This effect may be responsible for the better broiler performance reported with mash diet containing small particles based on hard wheat (Rose *et al.*, 2001; Pirgozliev *et al.*, 2003).

Gizzard development is strongly dependent on the feed particle size (Nir *et al.*, 1995; Engberg *et al.*, 2002). A coarse particle size stimulates better development of the gizzard, leading to increased digestion of grain. However, there is evidence that smaller particle size with an increased surface area improves access for digestive enzymes for the digestion of nutrients (Waldroup, 1997) and presumably lower energy expenditure on mastication (Jurgens, 1993).

Two key milling techniques, hammer and roller milling, are employed by the feed mill industry, and both have a bearing on the physical makeup of components of the grains (Little, 1997). Traditionally, the hammer mill is used to reduce the particle size of the grains, while roller milling has been applied mainly to produce coarser feeds (Koch, 1996; Waldroup, 1997). Roller mills can, however, produce fine particles of similar uniformity compared to those produced by hammer mills. In either case a range of particle sizes is produced depending on a number of factors, including the type of grain used, the speed of grinding and screen size used (Koch, 1996; McCracken, 2002), feed rate, power of the motor and speed of air flow through the mill (Martin, 1985). It must be noted that even within a grain type, grinding in the same mill type under similar conditions may result in different particle sizes (Lentle *et al.*, 2006) due to variations in endosperm hardness.

There are reports that chickens fed on maize-based diets ground with a roller mill had superior weight gain and feed conversion ratio compared to chickens fed same diet ground in a hammer mill (Reece *et al.*, 1985), however the results are not consistent (Douglas *et al.*, 1990). The influence of particle size appeared to be confounded by the complexity of the diet and nature of feed processing, such as milling, pelleting and crumbling (Goodband *et al.*, 2002). There is broad agreement that the uniformity of the diet is important for optimum performance of broilers, especially those raised in close confinement using automated feeding equipment (McCoy *et al.*, 1994).

The present study was designed to investigate the effects of milling technique and particle size on the nutritive value and feed utilization of the maize grain from different sources. The study also aimed to evaluate the effects of the milling technique and particle size on gastrointestinal physiology and performance of broiler chicken.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design and bird management

In the present study, a 2 x 2 x 3 factorial experiment was designed (Table 4.1) to investigate the effect of milling technique (hammer vs. roller) with differing particle size (fine or coarse) of maize from three sources (Downs, Emerald or Moree) on nutrient composition, growth performance, ileal digestibility and intestinal microbial population of broiler chickens up to 21 days of age. A total of 420 day-old male Cobb broiler chicks (Baiada Poultry Pty. Ltd., Tamworth, NSW, Australia), weighing 37.83 ± 0.04 g, were randomly allocated to 12 treatments with 5 replicates (7 birds per replicate) in brooder cages (600 × 420 × 23 cm) set up in an environmentally controlled room.

Table 4.1 Dietary treatments

	Hammer Mill (H)		Roller Mill (R)	
Maize source	Fine (<2 mm)	Coarse (2-4 mm)	Fine (<2 mm)	Coarse (2-4 mm)
Downs (DO)	DOHF=T1	DOHC=T2	DORF=T3	DORC=T4
Emerald (EM)	EMHF=T5	EMHC=T6	EMRF=T7	EMRC=T8
Moree (MO)	MOHF=T9	MOHC=T10	MORF=T11	MORC=T12

The grains used in this experiment came from three producing areas in NSW and QLD, Australia (harvested in 2007) and were obtained from commercial sources. Before diet preparation, a short screening test was conducted by sieving the grain through a 2 mm sieve in order to determine the particle size of grains of the three cultivars after grinding through a hammer mill (Jas Smith Pty Ltd, Ballarat, North VIC, Australia), which drives at about 2500 revolutions per minutes and a roller mill (Wolf Engineering & Millwrights Pty Ltd, Thomastown, VIC, Australia) (Table 4.2). There was no significant difference in the particle size produced by each milling technique between the three sources of maize.

The semi-purified diet used was a coarsely and finely ground maize-based diet without microbial enzyme supplementation. The finely ground diet was prepared using a hammer mill or roller mill with a 2 mm screen and, for the coarsely ground diet, a 4 mm screen was used as well.

Table 4.2 Particle size distribution of roller-milled and hammer-milled grain from three cultivars of maize passed through a 2 mm screen

Maize source	Particle size class (mm)	Hammer milling		Roller milling	
		Coarse particle (%)	Fine particle (%)	Coarse particle (%)	Fine particle (%)
Downs	>2	33	17	59	4
	< 2	67	83	41	96
Emerald	>2	34	17	58	5
	<2	66	83	42	95
Moree	>2	37	13	57	6
	<2	63	87	43	94

Over the entire experimental period (1–21d), the maize-based starter diet (Table 4.3) was fed to the birds. An indigestible marker (celite) was incorporated to assess the nutrient digestibility. The birds were brooded with an initial temperature of 33 °C which was reduced to 25 ± 1 °C gradually over 21 days. Sixteen hours of lighting was provided throughout the trial period. All birds were provided with the experimental diet as mash and water *ad libitum*. All feed samples were analyzed for crude protein by the method of Sweeney (1989) using a LECO® FP–2000 automatic nitrogen analyzer (Leco Corp., St Joseph, MI, USA).

On days 7 and 21, one and three birds, respectively were randomly selected from each replicate, weighed and humanly killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and subsequently removed. The contents of the gizzard, jejunum, ileum and caeca were collected into plastic containers for pH and digestibility (ileum content only) measurement. The ileal digesta were frozen immediately after collection and freeze-dried (Martin Christ Gerfriertrocknungsanlagen, GmbH, Osterode am Harz, Germany). They were then ground in a small coffee grinding machine and stored at –4 °C in airtight containers until chemical analyses were performed.

Table 4.3 Ingredient and nutrient composition of the diet fed

Ingredients	**g/kg**
Maize	722.7
Soycomil R (65 % CP)	218.7
Limestone (38 % Ca)	15.1
Vegetable oil	10.0
Dicalcium Phosphate	14.1
Sodium bicarbonate	4.6
Lysine- HCl	2.1
DL- Methionine	2.0
Salt	1.7
Choline Chloride (60 % Choline)	2.0
Broiler premix[1]	2.0
Celite	5.0
Nutrient composition (g/kg)	
ME poultry (MJ/kg)	12.8
Crude Protein	210.0
Lysine	13.2
Methionine	5.4
Arginine	13.6
Methionine + Cystine	8.8
Histidine	5.7
Threonine	8.5
Calcium	10.0
Available Phosphorus	4.2
Sodium	2.0
Choline	2.06

[1]Supplied per kg of diet (mg): vitamin A (as *all-trans* retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-calcium pantothenate, 12 mg; folic acid, 2 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg; and Mo, 1 mg.

4.2.2 Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No.: AEC 08/002). Health and animal husbandry practices complied with the *Code of Practice for the Use of Animals for Scientific Purposes* issued by the Australian Bureau of Animal Health (National Health and Medical Research Council, 1990).

4.2.3 Measurement and analyses

4.2.3.1 Growth performance

Feed intake (FI) and live weight (LW) on a cage basis were recorded at weekly intervals for determination of average FI and LW. Mortality was recorded as it occurred and feed conversion ratio (FCR; feed intake/weight gain) was corrected for mortality.

4.2.3.2 Visceral organ weight

The body weight and the weight of the proventriculus, gizzard and small intestine (the region from the distal end of the gizzard to 1 cm above the ileo-caecal junction) with contents, pancreas, bursa of Fabricius, yolk sac, spleen and liver were recorded at days 7 and 21. The relative organ weight was subsequently calculated as an indication of mass per unit of body weight (g/100 g of body weight).

4.2.3.3 Intestinal pH

Intestinal pH was measured immediately following death and excision of viscera at day 21. The pH of gizzard, jejunum, ileum and caecal contents was determined by modifying the procedure described by Corrier *et al.* (1990). Around 1 g of content was diluted in 9 mL of cold distilled water. The suspension was shaken vigorously with a stirrer and the pH was determined by insertion of a glass electrode (EcoScan 5/6 pH meter, Eutech Instruments Pte Ltd., Singapore).

4.2.3.4 Acid insoluble ash

The concentration of acid insoluble ash (AIA) in feed and freeze-dried ileal digesta was determined after ashing the samples and treating the ash with boiling 4 M HCl, following the methods described by Vogtmann *et al.* (1975), and Choct and Annison (1990). Samples (3 g of diet or 1 g of ileal digesta) were weighed accurately into Pyrex®-brand Gooch-type crucibles (porosity 4 μm) and dried (overnight at 105 °C) in a forced-air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd., Perth, Australia). After cooling and weighing, the samples were ashed overnight at 480 °C in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK). The crucibles were

placed in a boiling 4 M HCl bath so that the samples were wetted from underneath. Afterwards, the samples were gently boiled once in 4 M HCl for 15 min and the crucibles were dried overnight at 105 °C and weighed. The acid insoluble ash (AIA) content was calculated using the equation:

$$\text{AIA (g/kg DM)} = \frac{\text{(Crucible + Ash weight) – (Crucible weight)}}{\text{(Crucible + Dry sample weight) – (Crucible weight)}} \times 100$$

4.2.3.5 Digestibility coefficient of nutrients

The ileal digestibility of protein, gross energy and starch of feeds and freeze-dried ileal digesta was analysed and related to the concentration of the AIA. Diets and ileal digesta were analyzed for protein, gross energy and starch as described in Chapter 3 in sections 3.2.3.1 and 3.2.3.3. The digestibility coefficient of nutrient was calculated using the equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{Digesta nutrient (g/kg) / Digesta AIA (g/kg)}}{\text{Diet nutrient (g/kg) / Diet AIA (g/kg)}}$$

4.2.3.6 Particle size characteristics in gizzard content

The dry matter of gizzard contents at 21d of age was determined after collection from gizzard according to the gravimetric method mentioned in Chapter 3 in Section 3.2.3.1 under the subsection of proximate analyses. After that, the gizzard content on a cage basis was determined by sieving through a 2 mm screen.

4.2.3.7 Enumeration of gut microbial community

Fresh intestinal contents, weighing around 1 g, from the ileum and caeca were transferred into 15 mL MacCartney bottles containing 10 mL of anaerobic broth (see Appendix 1 for the composition). The suspension was homogenized for 2 min in CO_2-flushed plastic bags using a bag mixer (Interscience, St. Norm, France) and then serially diluted in 10-fold increments in anaerobic broth according to the procedure described by Engberg *et al.* (2004) and Miller and Wolin (1974). One millilitre of the homogenized suspension was then transferred into 9 mL of anaerobic broth and serially diluted from

10^{-1} to 10^{-5} for ileum samples and 10^{-1} to 10^{-6} for the caecal samples. From the last three diluted samples, 0.1 mL each was plated on the appropriate medium (10 mL) for enumeration of microbial populations.

Lactic acid bacteria were enumerated on MRS agar (Oxoid, CM0361) incubated under anaerobic conditions at 39 °C for 48 h. Coliform (red colonies) and lactose-negative *Enterobacteria* (colourless colonies) were counted on MacConkey agar (Oxoid, CM 0007) incubated aerobically at 39 °C for 24 h as red and collarless colonies, respectively. Lactobacilli were enumerated on Rogossa agar (Oxoid, CM 0627) after anaerobic incubation at 39 °C for 48 h. Total anaerobic bacteria were counted using anaerobic roll tubes containing 3 mL of Wilkins-Chalgren anaerobic agar (Oxoid, CM 0619) incubated at 39 °C for 7 days. Numbers of *C. perfringens* (*Cp*) were counted on Tryptose-Sulfite-Cycloserine and Shahidi-Ferguson Perfringens agar base (TSC & SFP) (Oxoid, CM 0587 OPSP) mixed with egg yolk emulsion (Oxoid, SR0047) and Perfringens (TSC) selective supplement (Oxoid, SR0088E) according to the pour-plate technique, where plates were overlaid with the same agar after spreading the inoculum and incubated anaerobically at 39 °C for 24 h. An anaerobic AnaeroGen™ sachet (AN0025A, Oxoid Ltd, Hampshire, UK) was used to generate the anaerobic environment (< 1 % O_2 and 9–13 % CO_2) for all anaerobically incubated agar plates.

The enumeration of microbial profiles was conducted only on chickens raised on diets containing fine hammer-milled and coarse roller-milled grains. This was done mainly to reduce the number of replicates analysed but the groups were chosen to reflect differences in particle size and milling techniques. Roller-milling at a coarse setting resulted in almost an equal balance in particle size while hammer-milling at a fine setting yielded predominantly fine particles (Table 4.2). After incubation, colonies formed on the respective media were carefully counted, converted into logarithmic equivalents ($\log_{10}$) and expressed as number of colony forming units (CFU) per gram of wet intestinal content.

4.2.4 Statistical analysis

Data for each day of sampling were analysed separately. The performance data such as FI, LW, FCR, relative weight of visceral organs, intestinal pH, nutrient digestibility and

gut microbial community were analysed using the General Linear Models (GLM) procedure of SPSS options, Version 17.0.0 (SPSS Inc, 2009) for the main effects of milling technique, particle size and source of grain, along with their interactions. Separation of means within a significant effect was done by Duncan's Multiple Range Test (DMRT) through post hoc procedure of SPSS. Significance levels were set at $P \leq 0.05$ unless otherwise specified.

4.3 RESULTS

4.3.1 Gross responses

Feed intake up to 7 days of age was affected by particle size but this was significant ($P<0.003$) only for maize from Downs that was roller milled, in which intake was higher in the diet containing finely milled grain than for the coarsely milled grain (Table 4.4). The interaction between milling technique and particle size on FI was also significant ($P<0.003$). In general, feed intake was higher in diets containing finely milled grain than coarsely milled grain (118.7 vs 112.6 g/bird). Live weight up to 7 days of age was affected by the source of maize but this was significant ($P<0.040$) for maize from Moree and Downs that was roller milled, in which live weight was higher in the diet containing finely milled grain than for the finely hammer milled Emerald maize. The interaction between milling technique and particle size was also significant ($P<0.002$). In particular, live weight was lower (109.0 g/bird) on diets containing Emerald maize than diets containing Moree and Downs (114.6 g/bird and 113.4 g/bird, respectively). There was no significant effect or interaction between maize source and milling technique on FCR up to 7 days of age. However, the FCR was marginally affected ($P<0.06$) by the interaction between milling method and particle size, with FCR tending to be improved by roller milling compared to hammer milling (1.53 vs 1.60 g:g). Feed conversion ratio was also better on coarse particle than fine particle diets (1.53 vs 1.60 g:g, respectively).

Table 4.4 Feed intake (FI), live weight (LW) and FCR of broiler chickens at 7 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments					
Source	**Milling**	**Particle**	**FI (g/bird)**	**LW (g/bird)**	**FCR (g:g)**
Downs	Hammer	Coarse	116.1^{abc}	116.3^{a}	1.48
		Fine	112.9^{abc}	111.3^{ab}	1.54
	Roller	Coarse	108.8^{bc}	108.7^{ab}	1.54
		Fine	124.4^{a}	117.2^{a}	1.57
Emerald	Hammer	Coarse	117.1^{abc}	110.5^{ab}	1.62
		Fine	117.4^{abc}	104.3^{b}	1.78
	Roller	Coarse	106.1^{c}	106.4^{ab}	1.55
		Fine	116.9^{abc}	114.6^{ab}	1.53
Moree	Hammer	Coarse	117.6^{abc}	115.6^{ab}	1.52
		Fine	120.6^{ab}	111.5^{ab}	1.67
	Roller	Coarse	109.8^{bc}	113.1^{ab}	1.46
		Fine	119.9^{ab}	118.4^{a}	1.50
Pooled SEM			*1.09*	*0.10*	*0.02*
Model P			*<0.01*	*<0.07*	*<0.09*
Source of variation					
Source			*NS*	*<0.040*	*NS*
Milling			*NS*	*NS*	*0.06*
Particle			*<0.003*	*NS*	*0.08*
Source × Milling			*NS*	*NS*	*0.09*
Source × Particle			*NS*	*NS*	*NS*
Milling × Particle			*<0.003*	*<0.002*	*NS*
Source × Milling ×Particle			*NS*	*NS*	*NS*

[1] Each value represents the mean of 5 replicates for each treatment group; [a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

Up to 21 days of age, there was no significant effect of maize source, milling technique, particle size or interaction between these factors on FI and LW (Table 4.5). However, LW was marginally higher (P<0.062) on the diet containing Moree maize (594.8 g/bird) than diets containing Downs (553.5 g/bird) and Emerald (531.7) maize. Feed conversion ratio up to this age was improved (P<0.042) on the Moree maize that was finely roller milled. The poorest FCR was observed in birds on the hammer milled Emerald maize diets.

Table 4.5 Feed intake (FI), live weight (LW) and FCR of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments					
Source	**Milling**	**Particle**	**FI (g/bird)**	**LW (g/bird)**	**FCR (g:g)**
Downs	Hammer	Coarse	845.8	597.2	1.51^{ab}
		Fine	851.5	606.7	1.50^{ab}
	Roller	Coarse	831.0	579.1	1.54^{ab}
		Fine	843.1	582.0	1.55^{ab}
Emerald	Hammer	Coarse	857.2	565.6	1.63^{a}
		Fine	827.8	546.4	1.63^{a}
	Roller	Coarse	822.5	568.6	1.55^{ab}
		Fine	820.0	597.9	1.47^{b}
Moree	Hammer	Coarse	871.4	596.7	1.56^{ab}
		Fine	862.1	584.0	1.58^{ab}
	Roller	Coarse	879.3	610.6	1.54^{ab}
		Fine	812.5	587.8	1.48^{b}
Pooled SEM			*6.94*	*4.73*	*0.01*
Model P			*NS*	*NS*	*NS*
Source of variation					
Source			*NS*	*0.062*	*NS*
Milling			*NS*	*NS*	*NS*
Particle			*NS*	*NS*	*NS*
Source × Milling			*NS*	*NS*	*<0.042*
Source × Particle			*NS*	*NS*	*NS*
Milling × Particle			*NS*	*NS*	*NS*
Source × Milling × Particle			*NS*	*NS*	*NS*

[1] Each value represents the mean of 5 replicates for each treatment group; [a, b] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

4.3.2 Visceral organ weight

At day 7, there was no significant effect of maize source, milling technique, particle size or interaction between the factors on the relative weight of small intestine (Table 4.6). However, the relative weight of small intestine was marginally increased (P<0.059) due to interaction between milling technique and particle size. The relative weight of pancreas was affected by an interaction between maize source and milling technique but this was significant (P<0.015) only when the group on finely milled Downs maize was compared to the group on Emerald, when both sources were hammer milled. The relative weight of liver was highest (P<0.004), in the group on the finely roller milled

Moree maize and lowest on diet with Downs maize that was hammer milled to a fine texture or coarsely roller milled.

There was a significant effect of particle size on the relative weight of proventriculus plus gizzard but this was significant (P<0.010) only when the groups on coarse roller milled Moree maize was compared to the group on finely milled (roller) Downs. In general, the relative weight of proventriculus plus gizzard was higher in diets containing coarsely milled grain than finely milled grain (8.5 vs 7.9 g/100 g of body weight).

The interaction between milling technique and particle size on the relative weight of proventriculus plus gizzard was also significant (P<0.007). In addition, there was a significant three-way interaction between the effect of sources, milling technique and particle size on relative weight of spleen. On diets based on Downs and Emerald, spleen weight was reduced when the hammer milled, fine diet was fed. For Moree, the fine roller milled diet caused this reduction. There were no significant effects of factors on relative weight of bursa of Fabricius and residual yolk sac at 7 days of age. However, the relative weight of yolk sac was slightly affected (P<0.091) by source and milling technique. The relative weight of yolk sac was highest in chickens on diets containing finely roller milled maize from Emerald.

At 21 days of age, there was no significant effect of maize source, milling technique, particle size or interaction between the factors on relative of weight of small intestine and proventriculus plus gizzard (Table 4.7). However, there was an effect of milling technique and particle size on the relative weight of pancreas but this was significant (P<0.01) only for chickens on maize from Emerald that was hammer milled, in which the relative weight of pancreas was higher in the diet based on coarsely milled grain than finely milled grain. Generally, the relative weight of liver was higher (P<0.01) in chickens raised on diets containing coarse particle compared to those on fine diets (3.7 vs 3.2 g/100 g of body weight). In addition, the relative weight of the liver was marginally affected (P<0.07) by milling technique, being higher in chickens on roller milled diets than on hammer milled diets. The relative weight of the bursa was significantly affected (P<0.02) by particle size, with generally higher values in chickens fed the finely milled diets than those coarsely milled diets (0.22 vs 0.19 g/100 g of body weight).

Table 4.6 Relative weight of visceral organs (g/100g of body weight) of broiler chickens at 7 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments									
Source	**Milling**	**Particle**	**Small Intes[2]**	**Pancr-eas**	**Liver**	**Pro+ Gizz[3]**	**Spleen**	**Burs -a**	**Yolk sac**
Downs	Hammer	Coarse	10.0	0.51^{abc}	4.4^{ab}	8.1^{abc}	0.08^{ab}	0.12	0.16
		Fine	11.9	0.59^{a}	4.1^{b}	8.5^{abc}	0.07^{b}	0.13	0.03
	Roller	Coarse	10.9	0.47^{bc}	4.2^{b}	8.8^{abc}	0.08^{ab}	0.13	0.09
		Fine	10.7	0.51^{abc}	4.9^{ab}	7.5^{c}	0.10^{ab}	0.15	0.09
Emerald	Hammer	Coarse	10.0	0.46^{c}	4.9^{ab}	7.9^{abc}	0.08^{ab}	0.14	0.04
		Fine	11.1	0.53^{abc}	4.4^{ab}	7.6^{bc}	0.11^{a}	0.15	0.03
	Roller	Coarse	11.1	0.56^{abc}	4.4^{ab}	8.9^{ab}	0.10^{ab}	0.13	0.06
		Fine	10.1	0.58^{ab}	4.7^{ab}	7.9^{bc}	0.07^{b}	0.13	0.34
Moree	Hammer	Coarse	9.2	0.52^{abc}	4.9^{ab}	8.0^{abc}	0.08^{ab}	0.15	0.10
		Fine	9.8	0.51^{abc}	4.3^{b}	7.9^{abc}	0.09^{ab}	0.15	0.07
	Roller	Coarse	10.9	0.54^{abc}	4.7^{ab}	9.3^{a}	0.07^{b}	0.14	0.07
		Fine	10.9	0.56^{abc}	5.3^{a}	7.7^{bc}	0.09^{ab}	0.14	0.07
Pooled SEM			*0.21*	*0.01*	*0.08*	*0.13*	*0.01*	*0.01*	*0.02*
Model P			*NS*	*NS*	*NS*	*<0.07*	*NS*	*NS*	*NS*
Source of variation									
Source			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Milling			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Particle			*NS*	*0.08*	*NS*	*<0.01*	*NS*	*NS*	*NS*
Source × Milling			*NS*	*<0.02*	*NS*	*NS*	*NS*	*NS*	*0.09*
Source ×Particle			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Milling ×Particle			*0.06*	*NS*	*<0.01*	*<0.01*	*NS*	*NS*	*NS*
Source × Milling × Particle			*NS*	*NS*	*NS*	*NS*	*<0.03*	*NS*	*NS*

[1] Each value represents the mean of 5 replicates for each treatment group; [a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); [2] Small intestines with digesta; [3] Proventriculus and gizzard with digesta; NS = Non-significant; SEM = Standard error of mean.

Table 4.7 Relative weight of visceral organs (g/100g of body weight) of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources[1]

Treatments								
Source	**Milling**	**Particle**	**Small intes.[2]**	**Pancreas**	**Liver**	**Pro+ gizz[3]**	**Spleen**	**Bursa**
Downs	Hammer	Coarse	6.8	0.36^{abc}	3.5	2.9	0.08	0.18
		Fine	6.1	0.34^{bc}	3.1	2.8	0.10	0.21
	Roller	Coarse	6.1	0.33^{bc}	4.0	2.9	0.11	0.21
		Fine	6.0	0.38^{abc}	3.0	2.7	0.11	0.23
Emerald	Hammer	Coarse	6.1	0.42^{a}	3.4	3.0	0.09	0.19
		Fine	5.9	0.34^{bc}	3.3	2.6	0.09	0.21
	Roller	Coarse	6.7	0.35^{abc}	4.1	2.9	0.10	0.18
		Fine	6.4	0.39^{abc}	3.6	2.7	0.12	0.23
Moree	Hammer	Coarse	6.4	0.34^{bc}	3.4	2.9	0.12	0.18
		Fine	5.8	0.31^{c}	3.1	3.0	0.10	0.22
	Roller	Coarse	6.2	0.37^{abc}	3.6	3.1	0.09	0.18
		Fine	6.4	0.41^{ab}	3.2	2.7	0.10	0.21
Pooled SEM			*0.09*	*0.01*	*0.08*	*0.06*	*0.00*	*0.01*
Model P			*NS*	*0.07*	*0.07*	*NS*	*NS*	*NS*
Source of variation								
Source			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Milling			*NS*	*NS*	*0.07*	*NS*	*NS*	*NS*
Particle			*NS*	*NS*	*<0.01*	*NS*	*NS*	*<0.02*
Source × Milling			*NS*	*NS*	*NS*	*NS*	*<0.02*	*NS*
Source × Particle			*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Milling × Particle			*NS*	*<0.01*	*NS*	*NS*	*NS*	*NS*

[1]Each value represents the mean of 5 replicates for each treatment group; [a, b, c] Values with unlike superscripts within each column are significantly different ($P<0.05$); [2]Small intestines weight considered with digesta; [3]Proventriculus plus gizzard weight considered without digesta; NS = Non-significant; SEM = Standard error of mean.

4.3.3 Intestinal pH

The pH of the intestinal contents is presented in Table 4.8. There were no significant effect of maize source, milling and particle size or interactions between these factors on the pH value of the intestinal contents from the gizzard, ileum and caecum at 21 days of age.

Table 4.8 The pH values of small intestinal content of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize[1]

Treatments					
Source	**Milling**	**Particle**	**Gizzard**	**Ileum**	**Caecum**
Downs	Hammer	Coarse	3.1	6.7	6.9
		Fine	3.0	6.8	6.8
	Roller	Coarse	3.1	6.3	7.0
		Fine	3.2	6.6	7.0
Emerald	Hammer	Coarse	3.3	6.9	6.9
		Fine	3.0	6.6	6.8
	Roller	Coarse	3.3	6.5	7.0
		Fine	3.1	6.8	6.7
Moree	Hammer	Coarse	2.9	6.8	6.8
		Fine	3.1	6.6	6.7
	Roller	Coarse	2.9	6.5	7.1
		Fine	3.1	6.9	6.9
Pooled SEM			*0.04*	*0.07*	*0.04*
Model P			*NS*	*NS*	*NS*
Source of variation					
Source			*NS*	*NS*	*NS*
Milling			*NS*	*NS*	*NS*
Particle			*NS*	*NS*	*NS*
Source × Milling			*NS*	*NS*	*NS*
Source × Particle			*NS*	*NS*	*NS*
Milling × Particle			*NS*	*NS*	*NS*
Source × Milling × Particle			*NS*	*NS*	*NS*

[1] Each value represents the mean of 5 replicates for each treatment group;
NS = Non significant; SEM = Standard error of mean

4.3.4 Nutrient digestibility

The ileal digestibility of protein was not affected by maize source and milling technique but this variable was affected (P<0.01) by particle size, in which digestibility of protein was higher in diets containing finely (0.82) milled grain than coarsely (0.79) milled grain (Table 4.9). There was a significant effect of maize source (P<0.01) and particle size (P<0.03) but not of milling technique on gross energy digestibility, with higher values in chickens raised on diets based on Emerald and Moree maize (0.76) than those on Downs (0.72). In general, gross energy digestibility was higher on diets containing finely milled grain than coarsely milled grain (0.76 vs 0.73). There was no significant interaction between the main effects on gross energy digestibility. Generally, starch digestibility was higher (P<0.01) on diets containing maize from Emerald (0.96) and

Moree (0.97) than Downs (0.94). There was no significant interaction between the main effects on starch digestibility at 21 days of age.

Table 4.9 Ileal protein, gross energy and starch digestibility of broiler chickens at 21days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize[1]

Treatments					
Source	**Milling**	**Particle**	**Protein**	**Gross energy**	**Starch**
Downs	Hammer	Coarse	0.80	0.72	0.93
		Fine	0.81	0.76	0.95
	Roller	Coarse	0.77	0.68	0.94
		Fine	0.81	0.73	0.94
Emerald	Hammer	Coarse	0.79	0.72	0.97
		Fine	0.82	0.78	0.98
	Roller	Coarse	0.81	0.77	0.96
		Fine	0.83	0.77	0.95
Moree	Hammer	Coarse	0.79	0.75	0.97
		Fine	0.81	0.77	0.97
	Roller	Coarse	0.80	0.77	0.97
		Fine	0.82	0.76	0.96
Pooled SEM			*0.00*	*0.01*	*0.00*
Model P			*NS*	*<0.02*	*NS*
Source of variation					
Source			*NS*	*<0.01*	*<0.01*
Milling			*NS*	*NS*	*NS*
Particle			*<0.01*	*<0.03*	*NS*
Source × Milling			*NS*	*NS*	*NS*
Source × Particle			*NS*	*NS*	*NS*
Milling × Particle			*NS*	*NS*	*NS*
Source × Milling × Particle			*NS*	*NS*	*NS*

[1] Each value represents the mean of 5 replicates for each treatment group; NS = Non-significant; SEM = Standard error of mean.

4.3.5 Particle size and dry matter of gizzard contents

The grain particle size characteristics of gizzard contents at 21 days of age are shown in Table 4.10. There was a significant effect of feed particle size (P<0.01) but not of maize source and milling technique on the proportion of particle sizes in gizzard contents. For birds fed finely ground diets, 26 % of the material found in the gizzard was less than 1 mm while for the coarsely ground diets, 19 % of the gizzard content was fine and the rest was coarse (>1 mm). The dry matter of gizzard contents was higher (P<0.01) on diets based on Moree (39.6) and Downs (38.6) maize than Emerald (36.2), and also

higher on coarse (38.8) than fine (37.4) particle diets. There was no significant interaction between main effects on particle size or dry matter of gizzard contents.

Table 4.10 Proportion of particle size classes in the gizzard content (on a dry weight basis) of broiler Chickens at day 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize[1]

Treatment					
Source	**Milling**	**Particle**	**Fine particles (< 1mm)**	**Coarse particles (> 1mm)**	**Dry matter (%)**
Downs	Hammer	Coarse	0.20	0.80	38.0
		Fine	0.23	0.77	38.7
	Roller	Coarse	0.16	0.84	40.0
		Fine	0.26	0.74	37.6
Emerald	Hammer	Coarse	0.21	0.79	36.2
		Fine	0.24	0.76	36.9
	Roller	Coarse	0.20	0.80	36.7
		Fine	0.26	0.74	35.1
Moree	Hammer	Coarse	0.19	0.81	41.2
		Fine	0.25	0.75	38.6
	Roller	Coarse	0.19	0.81	40.7
		Fine	0.31	0.69	37.8
Pooled SEM			*0.009*	*0.009*	*0.390*
Model P			*<0.04*	*<0.04*	*<0.03*
Source of variation					
Source			*NS*	*NS*	*<0.01*
Milling			*NS*	*NS*	*NS*
Particle			*<0.01*	*<0.01*	*0.05*
Source × Milling			*NS*	*NS*	*NS*
Source × Particle			*NS*	*NS*	*NS*
Milling × Particle			*NS*	*NS*	*NS*
Source × Milling × Particle			*NS*	*NS*	*NS*

[1] Each value represents the mean of 5 replicates for each treatment group; NS = Non significant; SEM = Standard error of mean.

4.3.6 Gut microflora

Overall, there was no significant effect of maize source, milling technique and particle size or interactions between these main effects on different types of bacterial populations in the ileal contents at 21 days of age (Table 4.11). However, the number of anaerobic bacteria (8.0 vs 7.7 $\log_{10}$ cfu/g digesta) and enterobacteria (4.2 vs 4.0 $\log_{10}$ cfu/g digesta) was marginally higher ($P<0.09$ and $P<0.08$, respectively) in chickens on diets containing coarsely roller milled grain than in those on finely hammer milled

grain. The number of *Clostridium perfringens* was numerically higher in the ileal content of birds on the roller-milled, coarse particle diet than those on hammer-milled, fine particle diet.

Table 4.11 Bacterial counts (log_{10} cfu /g digesta) in ileal digesta of broiler chickens at 21 days of age given finely and coarsely ground diets (maize based) obtained by hammer or roller milling from various sources of maize

Treatments						
Source	**Milling**	**Particle**	**Anaerobic**	**Lactic acid**	***C. perfringens***	**Entero-Bacteria**
Downs	Hammer	Fine	7.4	7.8	3.1	4.0
	Roller	Coarse	8.1	6.9	3.6	4.3
Emerald	Hammer	Fine	7.9	7.9	3.2	3.9
	Roller	Coarse	7.8	8.2	3.1	4.0
Moree	Hammer	Fine	7.9	8.2	3.0	4.1
	Roller	Coarse	8.2	8.3	3.3	4.2
Pooled SEM			*0.089*	*0.260*	*0.104*	*0.051*
Model P			*NS*	*NS*	*NS*	*NS*
Source of variation						
Source			*NS*	*NS*	*NS*	*NS*
Milling & Particle			*<0.09*	*NS*	*NS*	*<0.08*
Source × Milling & Particle			*NS*	*NS*	*NS*	*NS*

NS = Non significant; SEM = Standard error of mean.

In general, the bacterial populations in caecal contents were not affected by maize source, milling technique and particles or the interaction between these factors (Table 4.12). An exception was observed in chickens on the finely hammer–milled diets, where generally higher ($P<0.05$) population of *C. perfringens* than birds on the coarsely rolled grain.

Table 4.12 Bacterial counts (log_{10} cfu /g digesta) in caecal digesta of broiler chickens at 21 days of age given finely and coarsely ground maize based diets obtained by hammer or roller milling from various sources of maize

Treatments						
Source	**Milling**	**Particle**	**Anaerobic**	**Lactic acid**	***C. perfringens***	**Entero-Bacteria**
Downs	Hammer	Fine	8.7	9.1	7.4	6.1
	Roller	Coarse	8.7	9.3	7.2	5.7
Emerald	Hammer	Fine	8.7	9.0	7.5	6.0
	Roller	Coarse	8.6	9.1	7.3	5.3
Moree	Hammer	Fine	8.8	8.9	7.3	6.0
	Roller	Coarse	8.8	9.1	7.3	5.7
Pooled SEM			*0.051*	*0.062*	*0.122*	*0.063*
Model P			*NS*	*NS*	*NS*	*NS*
Source of variation						
Source			*NS*	*NS*	*NS*	*NS*
Milling & Particle			*NS*	*NS*	*<0.05*	*NS*
Source × Milling & Particle			*NS*	*NS*	*NS*	*NS*

NS = Non significant; SEM = Standard error of mean.

4.4 DISCUSSION

4.4.1 Gross responses

The present study revealed that the growth performance of broiler chicks was affected by the source of maize, particle size and their interactions in mash form. Nir *et al.* (1994b) found similar responses and stated that the sizes and texture of diets affect the performances of broiler chickens considerably. Feed intake increased significantly on the fine particle size diet up to day 7, but not at a later age. This may be due to undeveloped small beaks at early age and agree with Moran (1982a) and Portella *et al.* (1988), who suggested that the particle size of maize consumed by birds was related to beak development and the age of the birds.

Milling technique had no effect on overall performance, but the interaction between hammer milling and particle size affected FI, LW and LWG at seven days of age. This finding was contrary to the report of Reece *et al.* (1985), who observed that chickens fed with mash from diets containing maize ground with a roller mill grew faster and more efficiently than those fed on diets containing maize ground with a hammer mill. This may have been the result of a higher proportion of coarse particles in hammer-

milled diets compared to roller-milled diets. In addition, the sources of maize made differences in LW and LWG at an early age (1-7 days) of the birds fed with mash. This may be a result of compositional differences between the varieties, and this is supported by the findings of Cowieson (2005), who reported that the chemical composition and nutritional value of maize differs from batch to batch, resulting in a high degree of variation in its energy value for poultry.

4.4.2 Visceral organ weight

In the present study there was an impact of particle size on the relative weights of the proventriculus-gizzard, liver and bursa. In particular, there was an increase in the relative weight of the proventriculus/gizzard and liver in early age for chickens on coarse particle diets, while the relative weight of the immune organ, the bursa, was positively influenced by the fine particles of diets at grower stage. Similar results were recorded by Nir *et al.* (1994b) and Svihus *et al.* (1997), who acknowledged that gizzard weight of broilers became significantly greater when they were fed diets with coarser particles than when they were fed fine mash. In the present study, there was no significant impact of diet on gizzard size at a later age. Gizzard development can be achieved by manipulating the feed particle size of diets and this can be employed as a nutritional strategy (Nir *et al.*, 1995; Engberg *et al.*, 2002).

4.4.3 Particle size distributions in gizzard content

The distribution of grain particles in the gizzard contents was influenced by the source of maize, the proportion of coarse and fine particles and the DM content of the diet. Significantly, higher DM in gizzard contents was observed in birds fed diets based on the Downs and Moree maize than Emerald maize. This may be due to the initial moisture content of individual sources, or perhaps due to variation in the particle size distribution of the milled grain. The proportion of particle sizes in the gizzard reflected the original particle distribution of the diets used, confirming minimal pre-ventricular processing of diets by birds.

4.4.4 Nutrient digestibility

There was a significant effect of grain source on ileal digestibility of energy and starch, this being highest in birds on diets based on Emerald and Moree maize. This variation may be due to differences in chemical composition of grain from different sources. Moreover, protein and energy digestibility were increased in chicks fed on fine particle diets. These findings are in contrast to the report by Parsons *et al.* (2006), who found that feeding medium to coarse particle maize improved nutrient digestion; most likely due to better gizzard development and function. In this study, the higher CP and gross energy digestibility was influenced by particle size of diet, fine particle being more beneficial than coarse particle. It may be that smaller particle size increases the surface area available to enzyme activity, allowing more nutrients to be digested and absorbed through the small intestine. This finding is in support of those by Cumming (1994), and Forbes and Covasa (1995), who stated that the particle size of feed may affect the digestive tract of birds and consequently nutrient digestibility as well.

In this study, there was no significant effect of milling technique on nutrient digestibility, however, particle size appears to be more important. In a study by Roelof *et al.* (2001), milling method (hammer or roller) had no effect on the extent of starch digestion but enhanced its digestion rate, and maximum digestibility resulted from roller-milled maize. The differences in starch digestibility due to maize variety may be due to the variable starch composition of the grain sources.

4.4.5 Gut microflora

There was no significant change in bacterial populations in the gut of chickens due to maize source, milling method or particle size. However, the population of *C. perfringens* was affected by interactions between milling technique and particle size. This observation is supported to some extent by the study of (Engberg *et al.*, 2002) who mentioned that the structural properties of feed, grain particle size and feed formulation can influence the intestinal microflora of poultry. For example, an increase in retention time in the gizzard and more acidic gizzard pH may not only kill ingested enteric pathogens, but also increase the fermentation by symbiotic bacteria in the crop that act

as seed stock to colonize the lower digestive tract and competitively exclude pathogens (Engberg *et al.*, 2004).

In the current study, there was a significant reduction in population of *C perfringens* in caecal content due to roller milling and coarse particle size. This is expected as a well developed gizzard is associated with improvement in gut motility (Ferket, 2000) and may prevent pathogenic bacteria such as *C. perfringens* from entering the small intestine (Bjerrum *et al.*, 2005). Such diets may reduce the risk of coccidiosis and other enteric diseases (Engberg *et al.*, 2004; Bjerrum *et al.*, 2005).

There was a marginal increase in the populations of total anaerobic bacteria, and Enterobacteria in the ileal contents of chickens in this study. Feed particle size, particularly coarse particles, is generally known to influence the development of intestinal morphometry especially the gizzard. The structure of the feed itself can also influence the microflora of the intestinal tract of broiler chickens.

4.5 CONCLUSIONS

The present study indicates that source, milling and particle size of grains are important in mash diets in terms of bird performance, especially in early life. In particular, fine particle size resulted in an increase in FI and one of the maize source (Moree) enhanced LW at the same growth phase. However, FCR was improved on hammer milled diets in maize from Downs in the third week of age. Large particle size has an initial stimulatory effect on the gizzard and subsequent effects on the liver and bursa.

Overall, the nutritive value and digestibility of starch were higher (>95 %) in this experiment than under the commercial practice, where a fixed level of maize (72 %) was used in diet. It is not certain if these responses will remain the same, regardless of level of maize inclusion or supplementation with appropriate microbial enzymes. These are the subject of investigations in the next chapter.

CHAPTER 5 THE RESPONSE OF BROILER CHICKENS TO RISING LEVELS OF MAIZE GRAIN AND SUPPLEMENTATION WITH MICROBIAL ENZYME

5.1 INTRODUCTION

Maize is probably the most important cereal grain used for poultry feeding around the world. It is low in viscous NSPs, the major anti-nutritive factors present in temperate cereals such as barley, wheat and oats. These NSPs are the factors responsible for the lower productivity of birds on diets containing wheat and other temperate cereals. Recent research has shown that, depending on its source, there is a wide variability in the quality of maize and that the nutritive value of maize can be improved through addition of microbial enzymes to the diet (Cowieson and Ravindran, 2008). Therefore, it is important for the consistency of maize-based diets that specific exogenous enzymes are supplemented to improve the digestibility of starch, oil and protein, as well as amino acids.

Many enzymes have been found to be beneficial when added to poultry diets containing carbohydrate or a protein source with high levels of NSP such as wheat, barley, triticale or peas. It has been reported that multi-enzyme feed additives increase the utilization of protein/amino acids, energy and minerals of cereal grains by broilers containing wheat/maize based diets (Mathlouthi *et al.*, 2003; Wang *et al.*, 2005; Saleh *et al.*, 2006) leading to improved broiler performance, FCR, dressing weight and breast muscle (Mori *et al.*, 2007; Tony *et al.*, 2007). The enzymes widely used by the broiler industry are glycanases (xylanases and β-glucanases) that hydrolyse the NSP in some cereals and more recently, microbial phytases that target the phytate complexes in plant-derived ingredients. The effects of xylanases and β- glucanases are well established (Annison and Choct 1991; Bedford and Schulze, 1998), however, the corresponding information on the influence of enzyme mixtures with microbial phytase is limited. It is well recognized that the microbial community and its activity in the broiler intestinal tract is influenced by dietary composition. In this respect, the effect of different grains (Wanger

and Thomas, 1978; Mathlouthi *et al.*, 2002; Apajalahti *et al.*, 2004) and also exogenous enzymes (Engberg *et al.*, 2004; Jozeflak *et al.*, 2006) have been reported. It has been demonstrated that the anti-nutritional effects of soluble cereal NSPs for broilers is partially mediated via intestinal microbial activity (Choct *et al.*, 1996; Choct *et al.*, 1999b). However, the increased interest in the study of broiler gastrointestinal tract (GIT) microflora in recent years is due to the ban on in-feed antibiotic supplements.

Pancreatic and intestinal mucosal enzymes are required for the digestion of ingested nutrients. The activity of pancreatic (Sklan and Noy, 2000) and intestinal mucosal (Uni *et al.*, 1999) enzymes are well correlated with the body weight of birds. The activity of digestive enzymes of broiler chickens can be affected by age (Noy and Sklan, 1994; Uni *et al.*, 1998; Iji *et al.*, 2001), exogenous enzymes (Engberg *et al.*, 2004) and also the form (Gabriel *et al.*, 2003) and type of cereal grains (Almirall *et al.*, 1995) with different inclusion levels in the diet. A limited number of studies have examined the changes in intestinal microbial community as influenced by diet based on maize at different inclusion levels.

Avizyme 1502 (Danisco Animal Nutrition, UK), an enzyme product showing activities of amylase, xylanase and protease, has been increasingly used by the broiler industry in recent years. It promotes the breakdown of starch, cell walls, storage proteins and proteinaceous anti-nutritional factors (Troche *et al.*, 2007). Studies have also suggested that the addition of Avizyme may be beneficial in terms of growth performance and intestinal activity in diets based on maize and soybean meal (Wyatt *et al.*, 1997; Zanella *et al.*, 1999; Douglas *et al.*, 2000; Cafe *et al.*, 2002). A positive effect from the addition of enzymes on body weight gain of chickens was also established by Charlton, (1997), Schutte and Pereira (1998) and Chesson (2001). Cafe *et al.* (2002) also reported that feeding maize-based diets supplemented with exogenous enzymes (Avizyme) improved the body weight of broiler chickens of various ages when compared to unsupplemented diets. These researchers also observed that diets containing Avizyme yielded a greater amount of net energy as well. These authors, however, emphasized that the application of enzymes gives positive results more in very young chickens than in older birds.

Another important anti-nutritional factor present in maize is phytic acid, which binds minerals, proteins, lipids and starch (Thompson and Yoon, 1984) and reduces the

digestibility of these nutrients for poultry (Sebastiana *et al.*, 1997). The role of microbial phytase in poultry diets in increasing calcium and phosphorus availability has been reported in the scientific literature (Driver *et al.*, 2005; Rama Rao *et al.*, 2006; Narcy *et al.*, 2009). Studies have also shown a beneficial effect of phytase (1200 FTU/kg) addition (Thompson and Yoon, 1984) in broiler diets on ileal digestibility of metabolizable energy and total amino acids (Namkung and Leeson, 1999; Ravindran *et al.*, 1999). However, recent literature indicated that the benefits of phytase in broiler diets may not be felt when dietary Ca level is not at an optimum (Powell *et al.*, 2010).

It is an industry requirement to improve energy efficiency through the use of the right microbial enzymes at the proper doses. This strategy has proven to be highly successful in diets that are based on maize, wheat and barley (Bedford and Morgan, 1996; Bedford and Schulze, 1998; Cowieson, 2005). However, the recent use of exogenous enzymes in maize-based diets with an increasing level of maize has not received as much attention in the scientific literature.

Therefore, the current study was conducted using nutritionally adequate diets based on increasing levels of maize with or without dietary supplementation with microbial enzyme cocktail (Avizyme and Phyzyme), to determine the response of broiler chickens in terms of growth performance, nutrient digestibility, intestinal enzyme activities and the gut microflora community.

5.2 MATERIALS AND METHODS

5.2.1 Experimental design and bird management

The experiment was a 3 × 2 factorial design with six dietary treatment combinations. There were three inclusion levels of maize (MIL), 250 g/kg (LM), 500 g/kg (MM) and 750 g/kg diet (HM) and two levels of enzymes (enzyme and no enzyme). A total of 210 day-old male Cobb broiler chickens (Baiada Poultry Pty. Ltd., Kootingal, Tamworth, NSW, Australia), weighing 47.15 ± 0.22 g were randomly assigned to six treatments, each with five replicates, seven chickens in each replicate. Chickens were reared in multi-tiered brooder cages (600 × 420 × 23 cm) placed in a climate control room up to day 21, when the feeding trial ended. Feed and water were provided *ad libitum*. The

room temperature was initially set at 33 °C and successively reduced to 25 ± 1°C at 21 days. Sixteen hours of lighting per day was provided throughout the trial. Titanium dioxide (TiO_2) was incorporated at a rate of 5 g/kg diet as an indigestible marker, to enable assessment of nutrient digestibility.

One of the maize sources investigated in Chapters 3 and 4, Emerald obtained from a commercial source (2007/2008 harvest season), was used in this experiment. The diets (Table 5.1) were semi-purified, maize-based, with or without enzyme supplementation, Avizyme 1502 (0.5 g/kg) and Phyzyme XP (0.1 g/kg). Avizyme 1502 is an enzyme product containing purified xylanase, amylase and protease targeted towards markets in which maize and sorghum are the primary energy sources used in poultry diets. Phyzyme XP is a highly effective phytase feed enzyme that improves the digestibility of phosphorus and other nutrients in cereal grains (Cowieson, 2005). Other dietary ingredients were Soycomil R 65 % (as protein source), vitamin mineral premix and vegetable oil. All of the experimental diets were iso-nitrogenous and iso-energetic. The maize grain was ground using a hammer mill with a 4 mm sieve prior to mixing and the diets were given to the birds in mash form.

On days 7 and 21, two birds and four birds, respectively from each cage were randomly selected, weighed and killed by cervical dislocation. Subsequently, the abdominal cavity was opened and the small intestine was ligated and removed. The contents of the gizzard, jejunum, ileum and caeca were collected in plastic bottles for measurement of pH or nutrient digestibility. In addition, around 1 g of ileal and caecal contents were collected into pre-prepared McCartney bottles containing anaerobic broth (see Appendix 1) for enumeration of microbial populations and stored in a freezer at –20 °C until analyses. For the determination of nutrient digestibility, the digesta from the ileum were collected, pooled on a cage basis, homogenized and stored at –20 °C. Later, the samples were freeze-dried and then ground in a small coffee grinding machine and stored at –4 °C in airtight containers for chemical analysis of TiO_2, gross energy, starch and protein.

Table 5.1 Ingredient and nutrient composition (g/kg) of the diets fed

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Maize	250.0	250.0	500.0	500.0	750.0	750.0
Soyacomill R 65 %	289.0	289.0	252.0	252.0	212.0	212.0
Maize starch	419.0	419.0	210.6	210.6	0.0	0.0
Choline chloride	1.0	1.0	1.0	1.0	13.0	13.0
Sodium bicarbonate	1.0	1.0	1.0	1.0	17.0	17.0
Limestone	13.0	13.0	13.0	13.0	1.0	1.0
Dicalcium Phosphate	15.0	15.0	15.0	15.0	1.8	1.8
Salt	3.0	3.0	3.0	3.0	1.5	1.5
L-Lysine	0.1	0.1	0.9	0.9	3.0	3.0
DL Methionine	1.9	1.9	1.7	1.7	1.0	1.0
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[1]	2.0	2.0	2.0	2.0	2.0	2.0
Avizyme 1502	0	0.5	0	0.5	0	0.5
Phyzyme XP	0	0.1	0	0.1	0	0.1
Nutrient composition (g/kg)						
ME poultry (MJ/kg)	13.0	13.0	12.9	12.9	12.8	12.8
Crude Protein	212.0	212.0	212.0	212.0	209.0	209.0
Lysine	13.0	13.0	12.9	12.9	12.8	12.8
Methionine	5.0	5.0	5.0	5.0	4.9	4.9
Arginine	13.1	13.0	12.9	13.2	13.4	12.9
Methionine+cystine	7.4	7.5	7.9	8.1	8.2	7.8
Histidine	3.8	3.9	4.1	3.8	3.9	4.2
Threonine	8.7	8.7	8.5	8.5	8.2	8.2
Calcium	10.0	10.0	10.0	10.0	9.9	9.9
Available Phosphorus	4.0	4.0	4.1	4.1	4.2	4.2
Sodium	1.8	1.9	2.0	1.9	2.0	1.8
Choline	1.6	1.5	1.7	1.5	1.6	1.5

[1]Supplied per kg of diet (mg): vitamin A (as *all-trans* retinol), 3.6 mg; cholecalciferol, 0.09 mg;vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-calcium pantothenate, 12 mg; folic acid, 2 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg; and Mo, 1 mg.

5.2.2 Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No.: AEC 08/002). Health and animal husbandry practices complied with the *Code of Practice for the Use of Animals for Scientific Purposes* issued by the Australian Bureau of Animal Health (National Animal Health and Medical research Council (1990).

5.2.3 Measurement and analyses

5.2.3.1 Growth performance

Feed intake (FI), live weight (LW), FCR and mortality were assessed as described in Section 4.2.3.1.

5.2.3.2 Visceral organ weight

The weight of the small intestine and proventriculus plus gizzard with contents, pancreas, bursa of Fabricius, spleen and liver were recorded at day 21 as was the body weight of the bird from which they were excised as described in Section 4.2.3.2.

5.2.3.3 Intestinal pH

The pH of the intestinal contents from the gizzard, jejunum, ileum and caeca was measured as described in Chapter 4 (Section 4.2.3.3).

5.2.3.4 Tissue protein and digestive enzyme analysis

To assess the digestive enzyme activities and protein concentration, the jejunal tissue was processed as described by Shirazi-Beechey *et al.*(1991). The frozen tissue was weighed and cut into small pieces in an ice-cold buffer (100 mM mannitol, 2 mM HEPES/Tris, 7.1) and the mucosa was then stripped into the buffer using a vortex mixer at high speed for 1 min. After filtration through a Buchner funnel, the mixture was homogenized at medium speed (No 2, 13000 g/min) for 30 s on an Ultra Turrax T 25 Basic Homogenizer (IKA® Works, Wilmington, NC, USA). Afterwards, sub-samples of the homogenate were taken into Eppendrof tubes (Eppendrof South Pacific, North Ryde, Australia) and stored in a freezer (–20 °C) for enzyme analysis. The pancreas was processed in a similar manner to the jejunum except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used instead of buffer and the entire tissue was homogenized. The homogenate tissue was then centrifuged at high speed (30 000 g) for 20 minutes to obtain a supernatant on which the analysis was done according to Nitsan *et al.* (1974).

The specific activities of jejunal and pancreatic enzymes were assessed by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al.* (2001b). On the jejunal homogenates, the assays were conducted for mucosal protein content and activities of alkaline phosphatase (EC 3.1.3.1), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.10). For the pancreas, an assay was conducted for chymotrypsin amidase (EC 3.4.21.1). The specific activities of enzymes were measured according to the methods previously described for other species (Holdsworth, 1970; Serviere-Zaragoza *et al.*, 1997) after standardization for poultry. The concentration of protein in both the jejunal mucosa and pancreatic tissue was measured using the Coomassie dye-binding procedure described by Bradford (1976). All of the raw data for protein concentration were processed through the recommended Lowry Software (Mcpherson, 1985) before statistical analysis.

5.2.3.5 Titanium dioxide analysis

The marker, TiO_2, was used mainly because it is method to analyse and yields more consistent results than AIA. The TiO_2 content of the digesta and diet samples was measured according to the method of Short *et al* (1996). Around 0.1 g of the freeze-dried digesta or feed sample was ashed in a porcelain crucible for 13 hours at 580 °C and dissolved in 5 ml of 7.4 M sulphuric acid upon cooling. The samples were then gently boiled for approximately 20 min until completely dissolved. After cooling, the solution was poured quantitatively into a 50 mL volumetric flask through filter paper (Whatman 541, hardened ashless, 90 mm Ø Cat No. 1541 090, Whatman International Ltd Maidstone, England). After that, 10 mL of hydrogen peroxide (30 % v/v) were added to each flask and the contents diluted up to 50 mL with Milli-Q water and mixed properly through inversion to avoid bubbles. A typical orange colour then developed, which intensity was dependent on TiO_2 concentration. Aliquots of the solutions obtained and of similarly prepared standard solutions were analyzed using a Hitachi 150-20 UV spectrophotometer (Hitachi Science Systems Ltd., Ibaraki, Japan) by measuring the absorbance at 410 nm. The TiO_2 content, measured in mg/mL, was determined from the standard curve and converted to mg/g of the sample.

5.2.3.6 Ileal digestibility coefficient of nutrients

The concentration of protein, gross energy and starch in feed and freeze-dried ileal digesta were analyzed along with the indigestible titanium dioxide marker, and was used to calculate the digestibility coefficient. Diets and ileal digesta were analyzed for protein, gross energy and starch as described in Chapter 3 in Sections 3.2.3.1 and 3.2.3.3.

The digestibility coefficient for nutrients was calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{Digesta nutrient (g/kg DM)} / \text{Digesta } TiO_2 \text{ (g/kg DM)}}{\text{Diet nutrient (g/kg DM)} / \text{Diet } TiO_2 \text{ (g/kg DM)}}$$

5.2.3.7 Enumeration of gut microbial community

Gut microbial populations were enumerated in digesta contents of the ileum and caeca at 21 days of age, according to the procedure described by Engberg *et al.* (2004) and Miller and Wolin (1974) and highlighted in Chapter 4 (Section 4.2.3.7).

5.2.4 Statistical analysis

Data were analysed using the multiple regression options of SPSS, Version 17.0.0 (SPSS Inc, 2009) for the main effects of increasing MILs and enzyme supplementation (Morris, 1998). Data were also subjected to GLM analysis and reported where there was an interaction between MIL and microbial enzyme supplementation. Separation of means within a significant effect was done by Duncan's Multiple Range Test (DMRT) through post hoc procedure of SPSS. Significance levels were set at $P \leq 0.05$ unless otherwise specified.

5.3 RESULTS

5.3.1 Gross responses

Up to 7 days of age, there was a decline in feed intake as maize inclusion level (MIL) rose ($P<0.001$, $R^2 = 0.56$) in the diets (Table 5.2). However, FI increased with the

inclusion of enzymes (P<0.007, R^2 = 0.56) only on the LM diet. There was an increase in live weight with an increase in MIL (P<0.001, R^2 = 0.41) in diets and with the addition of microbial enzyme supplement (P<0.047, R^2 = 0.41). Overall, there was no any significant response in LW to changes in MIL with or without microbial enzyme supplement in early life. Up to this age, the FCR of the chicks was improved (P<0.001, R^2 = 0.61) as MIL increased in the diets while enzyme supplementation had no effect on FCR.

Table 5.2 Feed intake (FI), live weight (LW) and feed conversion ratio (FCR) of broiler chickens at days 7 at various maize inclusion levels (MIL) with or without microbial enzyme supplementation[1]

Treatments				
MIL (g/kg)	**Enzyme**	**FI (g/bird)**	**LW (g/bird)**	**FCR (g:g)**
250	–	79.0^{b}	98.8^{b}	1.62^{a}
	+	91.6^{a}	110.0^{ab}	1.47^{a}
500	–	71.3^{bc}	112.2^{a}	1.11^{b}
	+	78.8^{b}	121.0^{a}	1.06^{b}
750	–	68.7^{c}	119.3^{a}	0.96^{b}
	+	71.0^{bc}	119.4^{a}	0.98^{b}
Pooled SEM		*1.83*	*2.03*	*0.06*
Model P		*<0.001*	*<0.004*	*<0.001*
Source of variation				
MIL		*<0.001*	*<0.001*	*<0.001*
Enzyme		*<0.007*	*<0.047*	*NS*

[1]Each value represents the mean of 5 replicates for each treatment group; [a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

Up to 21 days of age, there was an increase in feed intake as MIL increased (P<0.001, R^2 = 0.57) in diets while supplementation with microbial enzyme (P<0.007, R^2 = 0.57) also improved feed intake only on the MM diet (Table 5.3). The interaction between MIL and enzyme on FI was also significant (P<0.050). There was an improvement (P<0.001, R^2 = 0.71) in LW in chickens with increase in MIL in diets. The microbial enzyme supplement also improved (P<0.004, R^2 = 0.71) LW but only on the MM diet. The interaction between MIL and enzyme on LW was also significant (P<0.020). The FCR was improved (P<0.001, R^2 = 0.42) in chickens with increase in MIL in diets, but the enzyme supplements had no effect on FCR up to day 21.

Table 5.3 Feed intake (FI), live weight (LW) and feed conversion ratio (FCR) of broiler chickens at days 21 at various maize inclusion levels (MIL) with or without microbial enzyme supplementation[1]

Treatments				
MIL (g/kg)	**Enzyme**	**FI (g/bird)**	**LW (g/bird)**	**FCR (g:g)**
250	–	642.5^{b}	426.5^{c}	1.70^{a}
	+	726.8^{ab}	489.8^{bc}	1.64^{ab}
500	–	706.6^{b}	506.6^{b}	1.54^{bc}
	+	814.0^{a}	583.8^{a}	1.52^{c}
750	–	824.8^{a}	594.0^{a}	1.51^{c}
	+	815.1^{a}	586.9^{a}	1.51^{c}
Pooled SEM		*15.47*	*12.83*	*0.02*
Model P		*<0.001*	*<0.001*	*<0.003*
Source of variation				
MIL		*<0.001*	*<0.001*	*<0.001*
Enzyme		*<0.007*	*<0.004*	*NS*
MIL × Enzyme		*<0.050*	*<0.020*	*NS*

[1]Each value represents the mean of 5 replicates for each treatment group; [a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

5.3.2 Visceral organ weight

At day 21, there was an increase (P<0.01, R^2 = 0.26) in relative weight of the small intestine with an increase in MIL, but this organ weight was not affected by the enzyme supplement (Table 5.4). There was no significant change in the relative weight of pancreas due to increase in MIL in diets with or without enzyme supplements at this age. There was an increase (P<0.03, R^2 = 0.31) in relative weight of the liver with increase in MIL in diets while enzyme supplementation also enhanced (P<0.02, R^2 = 0.31) liver weight only on the MM diet. There was an increase (P<0.01, R^2 = 0.27) in relative weight of the proventriculus plus gizzard with a rise in MIL in diets but there was no effect of enzyme supplementation on the weight of these organs. There was no significant change in the relative weight of spleen with increase in MIL in diets, with or without enzyme supplement. However, there was an increase (P<0.05, R^2 = 0.15) in the relative weight of bursa of Fabricius with the addition of microbial enzymes in diets, but no significant effect of MIL in diets.

Table 5.4 Relative weight of small intestine, proventriculus-gizzard, pancreas, liver spleen and bursa (g/100g of body weight) of broiler chickens at day 21 at various maize inclusion levels (MIL) with or without microbial enzyme supplementation[1]

Treatments							
MIL (g/kg)	**Enzyme**	**Small intest[2]**	**Pancreas**	**Liver**	**Pro+ Gizz[3]**	**Spleen**	**Bursa**
250	–	5.4	0.30	2.8^{c}	4.1^{b}	0.09	0.16
	+	5.5	0.35	3.0^{c}	4.1^{b}	0.10	0.20
500	–	5.9	0.33	3.3^{c}	4.6^{ab}	0.08	0.17
	+	6.5	0.32	4.1^{a}	5.1^{a}	0.09	0.20
750	–	6.3	0.33	3.1^{c}	4.6^{ab}	0.10	0.18
	+	6.8	0.37	4.0^{bc}	5.1^{a}	0.10	0.22
Pooled SEM		*0.18*	*0.01*	*0.14*	*0.13*	*0.00*	*0.01*
Model P		*NS*	*NS*	*<0.01*	*<0.04*	*NS*	*NS*
Source of variation							
MIL		*<0.01*	*NS*	*<0.03*	*<0.01*	*NS*	*NS*
Enzyme		*NS*	*NS*	*<0.02*	*NS*	*NS*	*<0.05*

[a, b, c] Values with unlike superscripts within each column are significantly different ($P<0.05$); [1]Each value represents the mean of 5 replicates for each treatment group; [2]Small intestine with digesta; [3] Proventriculus plus gizzard with digesta; NS = Non-significant; SEM = Standard error of mean.

5.3.3 Intestinal pH

At 21 days of age, the pH of the digesta in the gizzard declined ($P<0.01$, $R^2 = 0.22$) with an increase in MIL in diets (Table 5.5). However, the pH of the gizzard content was not changed by the enzyme supplementation. The pH value of the jejunal content marginally declined ($P<0.07$, $R^2 = 0.18$) with an increase in MIL in diets. There was no significant change in the pH value of digesta in ileum with an increase in MIL, with or without the enzyme supplements. There was an increase in the pH of the digesta in the caecum with increase in MIL ($P<0.02$, $R^2 = 0.34$) in the diets. The microbial enzyme supplement also enhanced ($P<0.01$, $R^2 = 0.34$) the pH value of caecal content but only on the HM diet.

Table 5.5 The pH values of small intestinal content of broiler chickens at day 21 at various maize inclusion levels (MIL) with or without microbial enzyme supplementation[1]

Treatments					
MIL (g/kg)	**Enzyme**	**Gizzard**	**Jejunum**	**Ileum**	**Caecum**
250	–	3.3	6.4	7.3	7.2^{d}
	+	3.2	6.6	7.5	7.5^{bcd}
500	–	3.0	6.4	7.2	7.8^{abc}
	+	3.1	6.4	7.1	7.9^{ab}
750	–	2.8	6.3	7.3	7.4^{cd}
	+	3.0	6.4	7.1	8.2^{a}
Pooled SEM		*0.06*	*0.03*	*0.08*	*0.08*
Model P		*NS*	*NS*	*NS*	*<0.01*
Source of variation					
MIL		*<0.01*	*0.07*	*NS*	*<0.02*
Enzyme		*NS*	*NS*	*NS*	*<0.01*

[1]Each value represents the mean of 5 replicates for each treatment group; [a, b, c, d] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

5.3.4 Tissue protein and enzyme activities

At 21 days of age, there was no significant change in pancreatic tissue protein content and chymotrypsin amidase activities with increase in MIL in diets, with or without enzyme supplement (Table 5.6). However, the protein content, as well as the activities of chymotrypsin amidase, tended to decrease with an increase in MIL, while the activity of chymotrypsin amidase was slightly increased in birds on the enzyme-supplemented diets. There was no significant change in tissue protein content and alkaline phosphatase activity in the jejunum but these tended to decline as MIL increased in diets. The activity of alkaline phosphatase marginally increased in birds fed on diets with enzyme supplementation but not significantly.

5.3.5 Nutrient digestibility

In general, there was no significant effect of MIL or enzyme supplementation on ileal digestibility of protein, gross energy and starch at 21 days of age (Table 5.7). However, digestibility slightly increased as MIL rose in diet and with exogenous enzyme inclusion.

Table 5.6 Tissue protein content and enzyme activities of broiler chickens at day 21 at various maize inclusion levels (MIL) with or without microbial enzyme supplementation[1]

Treatments		Pancreas		Jejunum	
MIL (g/kg)	Enzyme	Protein (mg/g tissue)	Chymotrypsin amidase[2]	Protein (mg/g tissue)	Alkaline phosphatase[3]
250	–	69.5	7.6	385.6	4.7
	+	70.9	6.8	350.7	5.6
500	–	53.5	7.3	319.8	4.2
	+	61.0	6.3	304.8	5.1
750	–	64.5	5.1	324.9	5.0
	+	59.1	6.4	346.2	5.3
Pooled SEM		*3.81*	*0.001*	*10.05*	*0.46*
Model P		*NS*	*NS*	*NS*	*NS*
Source of variation					
MIL		*NS*	*NS*	*NS*	*NS*
Enzyme		*NS*	*NS*	*NS*	*NS*

[1]Each value represents the mean of 5 replicates for each treatment group; [2]Specific activity (ηmol/mg protein/min); [3]Specific activity (μmol/mg protein/min); NS = Non-significant; SEM = Standard error of mean.

Table 5.7 Protein, gross energy and starch digestibility of broiler chickens at day 21 at various maize inclusion levels (MIL) with or without microbial enzyme supplementation[1]

Treatments				
MIL (g/kg)	Enzyme	Protein	Gross energy	Starch
250	–	0.82	0.79	0.97
	+	0.84	0.82	0.99
500	–	0.81	0.78	0.96
	+	0.84	0.79	0.98
750	–	0.86	0.82	0.98
	+	0.86	0.84	0.99
Pooled SEM		*0.008*	*0.011*	*0.004*
Model P		*NS*	*NS*	*NS*
Source of variation				
MIL		*NS*	*NS*	*NS*
Enzyme		*NS*	*NS*	*NS*

[1]Each value represents the mean of 5 replicates for each treatment group; NS = Non-significant; SEM = Standard error of mean.

Table 5.8 Bacterial counts (log_{10} cfu /g digesta) in ileum and caecum sections of digestive tract of broiler chickens given different levels of maize grain with or without enzyme-based diet fed as mash at day 21[1]

Treatments		Ileal content					Caecal content				
MIL (g/kg)	**Enzyme**	**Anaero-bic**	**Lactic acid**	**Lacto-bacilli**	***Entero bacteria***	***C. perfrin gens***	**Anaero-bic**	**Lactic acid**	**Lacto-bacilli**	***Entero bacteria***	***C. perfrin gens***
250	–	7.7	7.9	7.7	4.9	4.3	7.7	7.3	7.2	6.3	5.2
	+	7.3	7.4	7.2	4.5	4.1	9.1	8.9	8.5	8.1	6.0
750	–	7.4	7.6	7.6	5.0	4.1	9.2	9.0	8.8	8.1	6.3
	+	7.7	7.5	7.4	4.4	3.9	9.2	9.0	8.9	7.9	5.4
Pooled SEM		*0.10*	*0.11*	*0.10*	*0.16*	*0.06*	*0.47*	*0.46*	*0.45*	*0.44*	*0.37*
Model P		*NS*	*NS*	*NS*	*NS*	*0.091*	*NS*	*NS*	*NS*	*NS*	*NS*
Source of variation											
MIL		*NS*	*NS*	*NS*	*NS*	*<0.05*	*NS*	*NS*	*NS*	*NS*	*NS*
Enzyme		*NS*	*NS*	*NS*	*NS*	*<0.07*	*NS*	*NS*	*NS*	*NS*	*NS*

[1]Each value represents the mean of 5 replicates for each treatment group; NS = Non-significant; SEM = Standard error of mean.

5.3.6 Gut microflora

There was no significant change in the population of different types of bacteria in ileal contents with increase in MIL in diets with or without enzyme supplement at 21 days of age (Table 5.8). Nevertheless, the number of *C. perfringens* significantly decreased ($P<0.05$, $R^2 = 0.32$) with increase in MIL in diets. However, the population of this species was marginally increased ($P<0.07$, $R^2 = 0.32$) with the addition of microbial enzyme. In addition, the number of lactic acid bacteria marginally decreased with the increase in MIL, while all bacterial populations increased with the inclusion of exogenous enzyme, however, these differences was not statistically significant. There was no significant change in the population of the different bacteria in caecal content as a result of increase in MIL in diets with or without the enzyme supplement (Table 5.8). However, these bacterial populations slightly decreased with the enzyme-supplemented diets.

5.4 DISCUSSION

5.4.1 Gross responses

The results of this experiment demonstrate a significant improvement in FI, LW and FCR up to day 21 broilers on a diet with higher MIL (MM and HM). Addition of enzyme also improved overall productivity in the LM and MM diets but not in the HM group. The MM diet with enzyme supplementation produced the best results in terms of bird performance. Overall, including the enzyme in the diet improved the growth of birds on this trial. This is partly in agreement with the results of Cafe *et al.* (2002), who found a significant improvement in body weight of male broilers at different ages with a nutritionally adequate broiler diet based on maize and soybean meal with supplementation of Avizyme (0.1 %). Shakouri *et al.* (2008) also reported a similar positive effect of supplemental enzyme on the performance of broiler chickens fed on a wheat-based diet. However, results from the current study contrast with the findings of Fathabadi *et al.* (2006), who did not observe any significant effect of Avizyme 1502 on FI, LW and LWG but improved FCR was recorded in Hubbard Classic chickens. A similar performance trial conducted by Zanella *et al.* (1999), found that enzyme

supplementation of identical basal diets improved LW and the FCR by 1.9 and 2.2 %, respectively. Maize may respond to carbohydrase enzyme supplementation due to presence of non-starch polysaccharides, a large part of which is arabinose and xylose (Chesson, 2001).

5.4.2 Visceral organ weight

Organ weight data revealed that inclusion of maize at high level increased the size/volume of the small intestine and liver, leading to improved digestive activity. Enzyme addition also increased the size of liver. The relative weights of the major digestive and immune organs were affected by MIL and enzyme supplementation. The significant enlargement of the small intestine and proventriculus/gizzard of birds on the higher MIL may be partly attributed to the influence of grain texture, resulting in stimulation of the digestive organs. This finding is in agreement with that of Zarghi and Golian (2009), who reported an increase in the weight of the gizzard and small intestine with an increase in triticale in the diet when measured at 18 and 42 days of age.

In this study, the relative weights of the digestive organs were not affected by supplementation of exogenous enzymes except for the liver, which was enhancement in birds fed on the diet supplemented with enzymes. This is in contrast to the findings of Omojola and Adesehinwa (2007) who reported that the inclusion of an exogenous enzyme, Roxazyme G, at different doses did not influence the relative weights of the gizzard and liver in broiler chickens. In an another study, Sayyazadeh *et al.* (2006), who noticed that dietary enzyme had no significant effect on the gizzard and liver weight, carcass yield and abdominal fat contents, but positively influenced the weight of the intestine in broiler chickens on a maize-based diet. These differences may be due to the nature of enzymes used in the different studies.

5.4.3 Tissue protein and enzymes activities

In this study, the MIL or enzyme supplementation had no effect on pancreatic and jejunal tissue protein content or mucosal digestive enzyme activities in broiler chickens. Intestinal enzyme activities are stimulated by chyme passing through the digestive tract (Duke, 1986). In a previous study on broiler chickens, Jiang *et al.* (2008) found an

increased activity of trypsin within the intestinal lumen, while lipase, pancreatic protease and trypsin activities were not affected by exogenous amylase levels. There was a slight reduction in jejunal protein content due to supplementation with microbial enzymes. This is in agreement with the findings of other researchers (Danicke *et al.*, 2000a; Iji *et al.*, 2004) and may have a positive effect on the use of nutrients. The cost of mucosal regeneration and nutrients may be re-directed to body growth.

5.4.4 Nutrient digestibility

It is generally known that the addition of exogenous enzymes to wheat/and maize-based diets increases digestibility of nutrients in broiler chickens (Zanella *et al.*, 1999; Scott *et al.*, 2003). However, in the current study, there were no significant changes in ileal nutritient digestibility due to MIL or enzyme supplementation or their interactions in broiler chicken at 21 days of age. Nevertheless, the digestibility of CP and gross energy was slightly increased on the HM diets when compared to lower MIL diets. In another study with broiler chickens, Zanella *et al.* (2004) found that enzyme supplementation allowed better nutrient digestibility in corn-soybean meal based diets. Cowieson *et al.* (2006c) also suggested that the digestibility of nutrients by broilers fed on maize-soybean meal-based diets can be improved by the use of a combination of xylanase, amylase, protease and phytase. The nature of the diet (among others, particle size, pellet or mash) may influence nutrient digestibility.

5.4.5 Gut microflora

The slight increase in the populations of total anaerobic bacteria and lactobacilli in the ileum of birds fed on a high MIL diet with supplemental enzyme may suggest a positive effect of substrate availability for microbial growth especially at a higher maize level. There was a slight increase in the number of of *C. perfringens* in the ileum and caeca. *Clostridium perfringens* is considered to be the causitive agent of necrotic entiritis and is a very active species in the hydrolysis of bile salts (Knarreborg *et al.*, 2002), which may depress broiler performance (Engberg *et al.*, 2000). However, in this study, there was no incidence of necrotic entiritis or depression in bird performance.

Generally, bacterial populations such as those of total anaerobic bacteria and lactobacilli are regarded as beneficial to the host and can be increased through enzyme supplementaion. Rosin *et al.* (2007) found a mixed effect on gut bacterial population; including a reduction in the number of *E. coli* due to an inclusion of enzymes. This effect may be dependent on the nature of the carbohydrate source and enzyme type.

5.5 CONCLUSIONS

The relatively higher level of maize (MM or HM) in diets caused a large and significant increase in FI (at a later age), LW and better FCR. The microbial enzyme supplementation also resulted in a significant increase in these variables but no change in FCR. Higher MIL has a positive effect on the relative weights of the small intestine, liver, proventriculus/gizzard and on intestinal pH, but had no effect on other visceral organs. Enzyme addition enhanced only the relative weight of the liver.

The results suggest that maize grain, particularly at relatively high dietary levels, when supplemented with appropriate exogenous enzymes, is beneficial to feed intake, live weight gain and FCR as well as the development of the GIT. It is therefore possible to save on expensive protein meals and use more maize than is currently the case in the industry.

However, it must be recognised that agronomic practices involved with maize production vary from one region to the other or even from year to year. Moisture content at harvest and drying practice are the most variable. The effects of these factors are examined in the next two Chapters.

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CHAPTER 6 EFFECTS OF HEAT TREATMENT AT VARYING TEMPERATURE ON NUTRIENT COMPOSITION AND ULTRA-STRUCTURE OF HIGH-MOISTURE MAIZE GRAIN

6.1 INTRODUCTION

In many countries, including Australia, maize is usually harvested either with high moisture content to minimize damage in the field or kept in the field for an extended period of time to dry naturally before harvesting. In the former situation the grain is subjected to artificial drying before storage or milling for inclusion in animal feed. This artificial drying may result in loss of quality such as increase in retrograde starch (resistant starch type 3; RS3) content (Brown, 1996). Retrograde starch is caused by high temperature heating of grains followed by storage at low temperature. Heat processing may also anneal the starch as the grains cool down. Improper natural or artificial drying has been linked to decreased starch yield, increased stress cracking, and kernel breakage (Peplinski *et al.*, 1994). The digestibility of cereal grains is influenced by the starch component, especially the ratio between amylose and amylopectin (McDonald *et al.*, 1995).

In the USA, maize is usually harvested at moisture contents of between 18 and 25 percent (FAO, 1992). However, periodic early frosts or wet autumn weather coupled with a producer's desire for timely harvest may necessitate harvest at a higher moisture level followed by high temperature drying. According to a US Grain Council producer survey (Anonymous, 2001) more than 50 per cent of on-farm maize drying takes place at temperature well over 70 °C with the starch gelatinization temperature. Artificial drying, in particular rapid drying of maize can cause damages such as brittleness. This is the most prevalent damage and is manifested in the form of recovered intact endosperm and generally reduces the number of large, premium grits produced in dry milling. Stress cracks also contribute to the breakage in maize during handling. Scorching and discoloration of maize characterize damage caused by overheating. This indirectly

contributes to the fragility of dried grains. Heat damages caused by excessive drying temperature not only result in physical damage to the kernel that affects milling properties, but also causes undesirable chemical changes that make starch and gluten separation difficult in wet milling (Stephen and Muhammad, 2008). Similar problems also face the US maize growers, the world largest maize producer. The US Grain Council (USGC) Advisory Team member (Burrack, 2009) recently informed the worldwide maize buyers that harvest has been much slower than usual on his Arlington, Iowa farms as a result of continuous rain showers in 2009. Wet harvesting can be done at around 30 % moisture, which reduces the quality of grain particularly when run through a dryer, a huge amount of fine materials can be created in it (Anonymous, 2001).

Due to the amorphous nature of amylopectin it is more readily digested than the amylose. The normal structure of starch granules from maize (spherical, 10-16 microns across) with protein bodies and matrix creates a favourable environment for enzymatic digestion (Taylor and Belton, 2002). This structure may be altered after artificial drying of high-moisture maize (HMM) grains, which may affect the nutritive value. There is a report that the crude protein of stack-burned yellow maize does not change much due to high temperature drying, however, there was a reduction in concentrations of various amino acids and increase in Maillard reaction products and reduction in *in vitro* digestibility due to such treatment (Panigrahi *et al.*, 1996).

In addition, the ratio between amylose and amylopectin is very important for the digestibility of maize starch. Noy and Sklan (1994) opined that about 15 % of maize starch is known to remain undigested up to the terminal ileum and is assumed to be resistant to digestion, which present an opportunity for use of exogenous enzymes as is done with wheat and other temperate cereals.

While most maize is sundried *in situ*, wet weather may necessitate the use of artificial drying. Panigrahi *et al.* (1996) observed that the artificial drying could result in more damage of starch. Long periods of sun drying have been known to cause stack-burn (loss of quality in the field). The quality of diets with stack-burned grains is low, tending to negatively affect broiler performance. It may therefore be beneficial to artificially dry maize even in parts of tropics. There is a need to determine the ideal

temperature and duration that would not negatively impact on the quality of the maize processed under such conditions.

The present study was carried out to investigate the effect of artificial drying of high moisture maize on the chemical composition and ultra-structure as well as the *in vitro* digestibility of the grains.

6.2 MATERIALS AND METHODS

6.2.1 Experimental design and drying methods

Maize grain of a single variety, from the 2009 planting year (at the end of April), was obtained from Inverell in northern New South Wales, Australia at around 23 % moisture. To eliminate any effects of hot-air drying and mechanical shelling on grain properties, maize was harvested by hand and split into four groups of approximately 120 kg each. One of the groups was dried in the sun for three days until the moisture content dropped to a constant level of about 13 %. The other three groups were dried artificially using a forced draught oven at 80, 90 or 100 °C for 24 hours. After the drying process, the warm samples were placed in paper bags and cooled at room temperature overnight. Upon cooling, the cobs were threshed with a Manual Corn Sheller and kept in sealed air-tight bag before grinding. The samples of all batches were ground with a hammer mill to pass through a 1 mm mesh screen and stored at 4 °C until analyses.

6.2.2 Laboratory analyses

The proximate analyses were done by the method of Association of Official Analytical Chemists (AOAC, 2002). Details of laboratory analyses for proximate composition, phytate-P, starch, NSP, amino acids, minerals and *in vitro* digestibility and viscosity were as described in Chapter 3 (Sections 3.2.3.1 to 3.2.3.10).

6.2.3 Electron microscopic images

The grains were scanned on a NeoScope, JCM-5000, table-top scanning electron microscope (JEOL Ltd, Tokyo, Japan). Whole grains were scoured around the edges

and cut in section, then mounted on the machine for assessment at a magnification of × 1000.

6.2.4 Statistical analysis

The data were subjected to non-parametric analysis using SPSS, Version 17.0.0 (SPSS Inc, 2009) followed by calculation of coefficient of variation (CV).

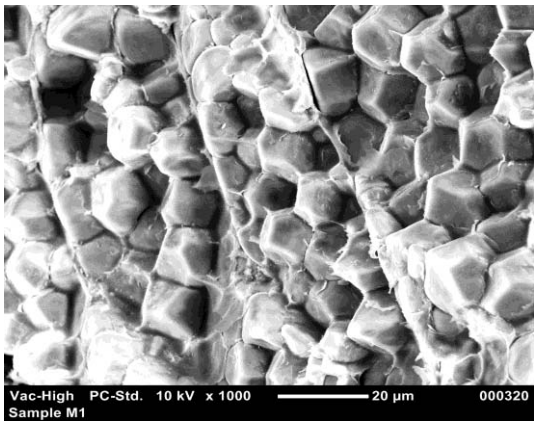
6.3 RESULTS

6.3.1 Ultra-structure of maize grains

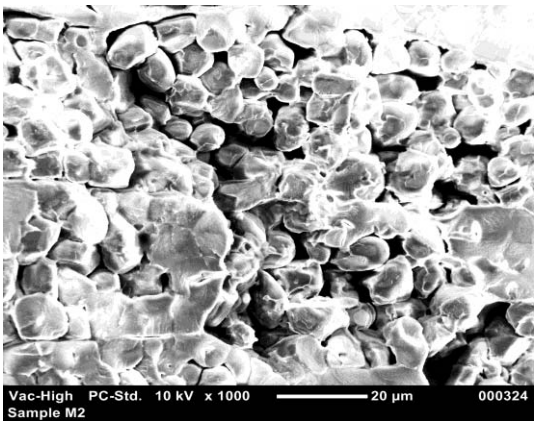
The scanning electron micrographs of the different grains revealed variations in the morphology of the starch granules (Plate 6.1). The granules shrunk in size as a result of artificial drying of the grains. The degree of shrinkage of granules was increased with increasing drying temperature. The internal matrix of the grains, which may reflect the linkage of starch and protein bodies, was also different. There was profound change in the size of starch granules, with excessive shrinkage, overlapping and matrix as well as reduced visibility of protein bodies in maize samples dried at 100 °C.

6.3.2 Proximate composition

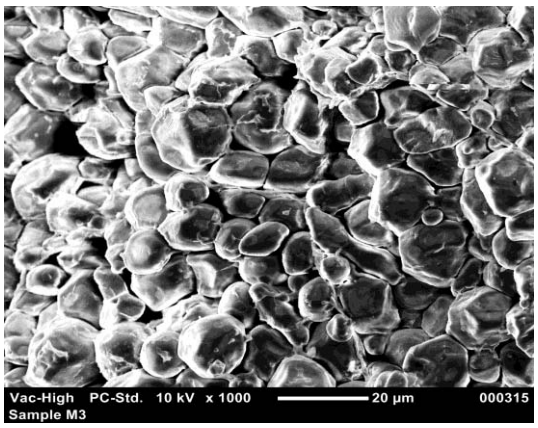
The proximate composition of the different batches of maize is shown in Table 6.1. The mean DM and ash contents of all grain batches varied between 870 and 980 g/kg, and 12.3 and 13.2 g/kg, respectively. The DM and ash contents were higher in artificially dried grains than in the sun-dried grain, however, there was not much difference (3.2 % and 2.3 %, respectively) in DM and ash content in the three heat-treated groups. The CP, and EE and phytate-P contents varied between 92.2 and 98.4; 41.3 and 45.0, and 1.21 and 1.80 g/kg, respectively in the samples dried under the sun and artificially at 80, 90 and 100 °C. These values were sharply decreased with increasing drying temperature, particularly, heat-treated maize had much lower values than sundried maize. The GE content was decreased by 2.1 %, while ME content, estimated from nutrient composition, was increased by 2.0 % when compared with sun-dried samples. The content of phytate-P was the most variable of the components in terms of CV.



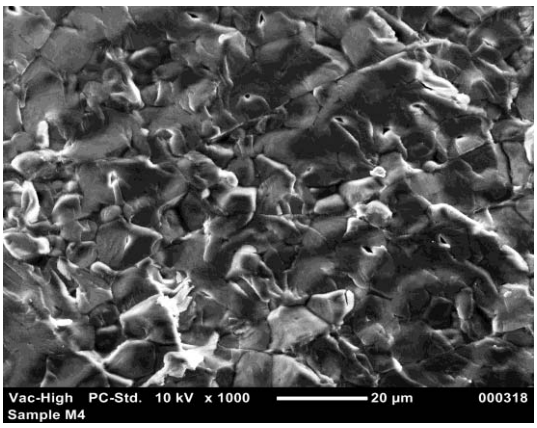
Sun-dried



Dried at 80 °C



Dried at 90 °C



Dried at 100 °C

Plate 6.1 Electron micrographs (× 1000) of maize dried under sun for three days (*top left*) and dried at 80 °C (*top right*), 90 °C (*bottom left*) and 100 °C (*bottom right*) for 24 hours

Table 6.1 Composition of maize batches observed under sun drying or artificially dried at different temperatures

Treatments	DM[1]	CP[1]	EE[1]	Ash[1]	Phytate P[1]	GE[2]	ME[2]
Sun drying	870.0	98.4	45.0	12.3	1.80	18.9	15.4
80 °C	950.0	93.4	42.0	12.9	1.36	18.4	15.6
90 °C	963.0	92.2	42.1	13.0	1.35	18.4	15.5
100 °C	980.0	93.8	41.3	13.2	1.21	18.5	15.7
CV	*00.05*	*0.03*	*0.04*	*0.03*	*0.18*	*0.01*	*0.01*

CV = Coefficient of variation; DM = Dry matter; CP = Crude protein; EE = Ether extract; GE = Gross energy; ME = metabolizable energy; [1] g/kg DM; [2]MJ/kg DM.

6.3.3 Starch content and composition

The total starch content varied between 670.1 g/kg in the sun-dried samples and 691.3 g/kg in samples dried at 80 °C (Table 6.2). Resistant starch and amylose contents were increased with increase in heating temperature, being highest in samples dried at 100 °C (415.7 g/kg and 313.8 g/kg, respectively). Compared to the sun-dried samples, the

contents of starch, RS and amylose were increased by 2.1, 31.3 and 14.3 %, respectively for the grain dried at 100 °C. In addition, the amylose and amylopectin ratio was also increased with increase in heating temperature and highest (0.46) in samples dried at 100 °C. Conversely, the content of amylopectin was reduced between 1.3 (90 °C) and 2.7 % (100 °C) when compared with sundried samples. The contents of resistant starch, amylose and the ratio of amylose: amylopectin were the most variable of the starch components.

Table 6.2 Starch content and components (g/kg DM) of maize batches after sun drying or artificial drying at different temperatures

Treatments	Starch	Resistant starch	Amylopectin	Amylose	Amylose: Amylopectin
Sun drying	670.1	316.6	482.3	187.8	0.39
80 °C	691.3	362.7	481.3	210.0	0.44
90 °C	687.8	366.3	476.0	211.8	0.44
100 °C	684.0	415.7	469.4	214.6	0.46
CV	*0.02*	*0.11*	*0.01*	*0.06*	*0.07*

CV = Coefficient of variation.

6.3.4 NSP and free sugar contents

Total soluble NSP content varied from 3.29 to 3.79 g/kg, being generally higher in the artificially dried grains than in the sun-dried samples (Table 6.3). Ribose, arabinose, xylose and galactose were the most variable of the component sugars, as revealed by the CV. There was very little variation in the concentrations of insoluble NSP, with CV generally lower than 0.05. In terms of free sugar contents, arabinose was increased with increase in heating temperature, being highest (0.39 g/kg) in samples dried at 100 ^{o}C. Other free sugar contents, for example; mannose, galactose, glucose and total free sugars decreased with increasing drying temperatures. Mannose and xylose were the most variable of these free sugar components, between the batches.

6.3.5 Amino acid contents

The amino acid composition of the maize batches is presented in Table 6.4. The concentrations of key amino acids such as methionine, threonine, alanine, phenylanaline, leucine, isoleucine and valine were increased by artificial drying

compared to sun drying. In addition, the concentration of some amino acids tended to increase with increase in drying temperature. However, lysine content was reduced by artificial drying. The methionine, lysine, leucine and isoleucine contents were the most variable amino acids between the groups.

Table 6.3 Non- starch polysaccharide (NSP) contents and composition of maize batches after sun drying or artificial drying at different temperatures

A. Soluble NSP content and components (g/kg DM)							
	Rib	**Ara**	**Xyl**	**Man**	**Gal**	**Glu**	**NSP**
Sun drying	0.05	0.55	0.35	1.89	0.34	0.50	3.29
80 °C	0.08	0.77	0.46	1.84	0.43	0.60	3.87
90 °C	0.07	0.76	0.46	1.86	0.40	0.58	3.69
100 °C	0.08	0.85	0.49	1.83	0.39	0.61	3.79
CV	*0.19*	*0.17*	*0.14*	*0.05*	*0.10*	*0.09*	*0.07*
B. Insoluble NSP content and components (g/kg DM)							
	Ara	**Xyl**	**Man**	**Gal**	**Glu**	**NSP**	
Sun drying	20.08	25.34	1.00	5.71	22.34	66.11	
80 °C	19.42	24.68	0.94	5.29	21.98	64.21	
90 °C	19.56	25.27	0.93	5.50	22.93	65.87	
100 °C	19.38	25.29	0.93	5.38	22.60	65.33	
CV	*0.02*	*0.01*	*0.04*	*0.03*	*0.02*	*0.01*	
C. Available free sugars content and components (g/kg DM)							
	Ara	**Xyl**	**Man**	**Gal**	**Glu**	**NSP**	
Sun drying	0.36	0.11	2.12	0.32	16.56	19.48	
80 °C	0.34	0.10	1.75	0.31	15.65	18.06	
90 °C	0.37	0.09	1.74	0.31	15.66	18.12	
100 °C	0.39	0.11	1.76	0.29	15.05	17.55	
CV	*0.06*	*0.09*	*0.10*	*0.04*	*0.04*	*0.05*	

CV = Coefficient of variation.

Table 6.4 Amino acid (g/kg DM) compositions of maize batches after sun drying or artificial drying at different temperatures

Treatments	**Met**	**Lys**	**Thr**	**Ala**	**Phe**	**Arg**	**Leu**	**Ile**	**Val**
Sun drying	1.2	2.2	3.0	6.4	4.3	4.2	10.3	3.3	4.1
80 °C	1.4	2.0	3.1	6.8	4.4	4.3	11.2	3.7	4.3
90 °C	1.4	1.9	3.2	6.9	4.6	4.2	11.7	3.6	4.4
100 °C	1.3	1.9	3.3	7.0	4.7	4.3	11.9	3.8	4.4
CV	*0.08*	*0.08*	*0.03*	*0.04*	*0.04*	*0.01*	*0.07*	*0.06*	*0.03*

CV = Coefficient of variation.

6.3.6 Mineral contents

There were lower concentrations of most micro elements except copper in the artificially dried samples than sundried maize (Table 6.5). Drying of maize at 100 °C resulted in lower manganese, zinc and iron contents than drying at 90 °C and 80 °C. The calcium, manganese, iron and copper contents were more variable between the batches than other minerals.

Table 6.5 Mineral contents of maize batches obtained by sun drying or artificially dried at different temperatures

	Macro elements (g/kg DM)				Micro elements (mg/g DM)					
Treatments	**Ca**	**P**	**Na**	**K**	**Mg**	**Mn**	**Zn**	**Fe**	**Cu**	**Mo**
Sun drying	0.05	2.9	7.9	3.5	1.6	6.2	17.0	37.4	1.0	0.43
80 °C	0.05	2.6	7.3	3.3	1.4	8.7	15.4	27.9	1.1	0.43
90 °C	0.04	2.5	7.1	3.1	1.3	5.1	14.0	25.2	1.1	0.39
100 °C	0.03	2.6	7.0	3.2	1.4	5.6	14.9	18.7	1.5	0.39
CV	*0.23*	*0.08*	*0.05*	*0.05*	*0.08*	*0.25*	*0.08*	*0.28*	*0.19*	*0.06*

CV = Coefficient of variation.

6.3.7 *In vitro* digestibility and viscosity

The mean *in vitro* dry matter, starch and protein digestibility values are shown in Table 6.6. The digestibility of these variables in all the grain batches varied between 48.0 and 52.5, 63.0 and 58.4, and 54.4 and 59.8, respectively. The digestibility of DM and protein were improved by artificial drying of the HMM but the starch digestibility was reduced. There was no effect of treatment on *in vitro* digesta viscosity.

Table 6.6 *In vitro* digestibility of dry matter, starch and protein as well as viscosity of maize batches after sun drying or artificial drying at different temperatures

Treatments	**Digestibility**			**Viscocity (cp)[1]**
	Dry matter	**Starch**	**Protein**	
Sun drying	48.0	63.0	54.4	0.92
80 °C	52.4	58.2	58.5	0.91
90 °C	52.3	57.6	55.4	0.91
100 °C	52.5	58.4	59.8	0.90
CV	*0.04*	*0.04*	*0.04*	*0.009*

[1] cP (centipoise) = 1/100 dyne second per centimeter squared; CV = Coefficient of variation.

6.4 DISCUSSION

In the present study, artificial drying of high moisture maize grains led to a considerable reduction in moisture content but variable changes in solid components. Drying at 100 ^{o}C tended to reduce the concentration of CP, GE and phytate-P. Similar findings were reported by Iji *et al*. (2003), who observed a reduction in the concentrations of ether extract and energy in maize grain due to drying grains at high temperature.

The reduction in phytic acid content through drying, may be useful as this chellate reduces the activity of pepsin, trypsin, and α-amylase, which are very important for digestion of key nutrients (Sebastiana *et al.*, 1998). However, artificial drying also resulted in an increase in the contents of most essential amino acids. This may have some implication for feed formulation as grains tend to be classified with the same nutrient compositions regardless of how they were dried.

Higher drying temperatures tended to increase the starch, resistant starch, amylose and amylose: amylopectin ratio of samples. Although, resistant starch is formed during seed development, its proportion may increase during feed processing, especially drying (Brown, 1996) and storage. While most maize is sun-dried *in situ*, wet weather may necessitate the use of artificial drying techniques. Artificial drying results in the annealing of starch, while long periods of sun drying have been known to cause stack-burn, a defect in quality of the crop (Panigrahi *et al.*, 1996).

The increase in amylose and its ratio with amylopectin, as well as increase in resistant starch in artificially heat-dried maize signify a decline in quality of grains (Ito *et al.*, 1999; Iji *et al.*, 2003). Energy content also decreased with heating, probably due to the volatilization of fat.

The extent of starch chain associations within amorphous regions and the degree of crystalline order are altered during heat treatment of HMM grains (Hoover and Vasanthan, 1994). The magnitudes of these changes, especially the varying proportions of amylose and amylopectin, were found to be dependent upon the moisture content during heat treatment and the starch sources.

In the present study, soluble NSP content was increased as a result of oven drying of maize grains. Soluble NSP are responsible for the reduction in nutritive value of diets based on temperate cereals but are generally low in maize and other tropical cereals (Choct and Annison, 1992). This may be responsible for the low viscosity of the *in vitro* digesta in this study.

Cereal grains are principally fed to provide energy and processing techniques must be designed to maximize total digestibility of the diet as a primary objective. The *in vitro* starch digestion was decreased as a result of artificial drying of the grains, and this may be due to increased amylose content. These findings are supported by those of Panigrahi *et al.* (1996). The increase in the content of resistant starch suggests a loss in grain quality since resistant starch is not responsive to animal enzymes (Noy and Sklan, 1994). On the other hand, these findings disagree with those of Nir *et al.* (1994a) who reported a 4.5 fold increase in *in vitro* digestion of maize starch by amylase after heat treatment due to increase in amylopectin content.

Different types of starch granules were observed in the different batches. The starch granules of maize are large and essentially spherical in shape in the fresh grains (Taylor and Belton, 2002). In the present study, most of the starch granules were squeezed, some of them overlapped each other and the granules were tightly packed in response to artificial drying. The size of the starch granule is an important factor in determining the energy value of grain, starch with smaller granules having a relatively larger surface area and so a greater potential for hydrolysis by endogenous amylase (Carre, 2004). During heat processing, the starch gelatinizes to an extent that is dependent on granule size, moisture content, amylose:amylopectin ratio and time (Klucinec and Thompson, 1999; Tester *et al.*, 2004).

6.5 CONCLUSIONS

From the present study it can be concluded that high moisture maize is affected by artificial drying and such treatment may change the chemical composition of the grains considerably. These effects were most pronounced at 100 °C. If it is necessary for high moisture maize to be artificially dried then drying temperature should be lower than 100

°C. The changes in starch composition may significantly affect the nutritive value of the grains. This is the subject of the next chapter and further investigations will be made into improvement of the heat-treated grains.

CHAPTER 7 RESPONSE OF BROILER CHICKENS ON DIETS BASED ON HIGH-MOISTURE MAIZE GRAIN SUBJECTED TO ARTIFICIAL DRYING AND SUPPLEMENTATION WITH MICROBIAL ENZYMES

7.1 INTRODUCTION

In many parts of the world, especially in humid areas, maize is harvested at relatively high moisture content, with a view to minimizing damage in the field when left to dry naturally. The grain is then subjected to artificial drying, which may result in loss of quality such as increase in retrograde starch (Brown, 1996). The retrograde starch is caused by high temperature heating of grains followed by storage at lower temperature. The digestibility of cereal grains is influenced by the starch component, especially the ratio between amylose and amylopectin (McDonald *et al.*, 1995). Noy and Sklan (1994) stated that about 15 % of maize starch is known to remain undigested up to the terminal ileum and is assumed to be resistant to digestion. This presents an opportunity for the use of exogenous enzymes as is done with wheat and other temperate cereals. The starch in high moisture grain also anneals after heat processing. It is not well known how much of these processes occur during routine production and processing of maize.

Due to the amorphous nature of its structure, amylopectin is more readily digested than amylose. Moreover, this readily available amylopectin content may increase due to artificial drying of high moisture grain and conversely amylose content may decrease. This was confirmed by Bhuiyan *et al.* (2010), but it is not known if further improvements could be achieved through supplementation with microbial enzymes.

The normal structure (spherical, 10–16 microns across) of starch granules with protein bodies and matrix may be altered easily and can create a favourable environment for enzymatic digestion (Taylor and Belton, 2002). However, the normal structure of starch granules may also change, becoming more shrunken if dried at high temperature, and

maize quality can be compromised (Bhuiyan *et al.*, 2010). The research of Panigrahi *et al.* (1996), who evaluated the effects of artificial drying of grains that had stack-burn and reduction in *in vitro* digestibility, showed that the quality of diets with stack burned/overheated maize grain tends to negatively affect broiler performance. There are many reasons advanced for these reductions in quality such as denaturation of heat-labile vitamins and damage to proteins via an interaction with reducing sugars (Maillard reaction) and retrograde starch formation after heat processing (Panigrahi *et al.*, 1996; Iji *et al.*, 2003; Cowieson, 2005).

Exogenous enzymes have been used for many years to break down cell walls in feed ingredients, to reduce the viscosity of digesta and to improve the digestibility of carbohydrates and proteins (Bedford and Morgan, 1996). Generally they are used to improve the nutritive value of wheat- and barley-based diets, but some enzyme preparations are also used to increase the nutritional value of maize and its by-products (Boros *et al.*, 2004). In addition, Cowieson (2005) reported that an enzyme preparation added to a diet based on maize improved feed conversion ratio by between 0.78 and 10.5 % and body weight gain from 0.5 to 10.9 % over the control. However, in some investigations no positive effect of enzymes was registered (Peric *et al.*, 2002; Iji *et al.*, 2003). Obviously the positive effect of enzyme preparation depends on the quantity and quality of feeds included in the mixture, energy level and type of enzymes, as well as the growth stage of chickens (Acamovic, 2001). There is little in the literature about the addition of enzyme mixture to diets based on artificially dried maize, especially if the grains were harvested with high moisture.

The aim of this trial was to evaluate the response of high-moisture maize grain to artificial drying and supplementation with microbial enzymes. These investigations were carried out from the points of feed intake, body weight and FCR, and the physiological mechanisms behind these responses.

7.2 MATERIALS AND METHODS

7.2.1 Experimental design and bird management

A 2 x 4 factorial experiment was performed to study the effect of drying high-moisture maize at various temperatures (sun-dried and artificially at 80, 90 or 100 °C), and with or without added microbial enzymes on nutritive value of the grain and physiological response of broiler chickens. A total of 384 day-old male Cobb broiler chicks (Baiada Poultry Pty. Ltd, Tamworth, NSW, Australia), weighing 40.06 ± 0.08 g, were randomly assigned to 48 cages (600 × 420 × 23 cm each) in four-tier battery brooders housed in an environmentally controlled house. Each of the 8 treatments was randomly assigned to 6 cages with 8 birds per cage. The birds were initially brooded at a temperature of 33 °C, but this was gradually reduced to 24 ± 1° C at 21 days of age when the feeding trial ended. Sixteen hours of lighting per day were provided throughout the trial period.

Maize grain obtained from Inverell in Northern New South Wales, Australia (2009 planting year; at the end of April) was used in this experiment. The maize was received at a moisture content of 23 % and split into four batches. One of the batches was dried in the sun for three days until the moisture content dropped to a constant level of about 13 %. The other three batches were dried artificially using a forced draught oven at 80, 90 or 100 °C for 24 hours. After drying, the dry matter content was at the level of 95.0, 96.3 and 98.0 %, respectively.

Four basal diets were formulated to meet the minimum National Research Council (NRC, 1994) nutrient recommendations for broiler chickens (Table 7.1). The experimental diets were semi-purified, comprising of more than 70 % of each maize type. Each of these diets was fed as such or supplemented with microbial enzymes at a rate of 0.5 g/kg of Avizyme 1502 (containing amylase 800, xylanase 1200, protease 8000 U/g) and 0.1 g/kg of Phyzyme XP (1000 FTU). Both of these enzymes were supplied by Danisco Animal Nutrition, UK. An indigestible marker, titanium dioxide, was incorporated in order to assess the nutrient digestibility of the given feed. The diets were formulated to be iso-energetic and iso-nitrogenous, and fed for 21 days. Feeds and water were available to the birds *ad libitum*.

Table 7.1 Ingredient and nutrient composition (g/kg) of the diets[1] fed

Ingredients	Diet 1 (sun-dried)	Diet 3 (80 °C)	Diet 5 (90 °C)	Diet 7 (100 °C)
Maize	71.0	72.6	72.6	73.1
Soycomil K (67 %)	22.6	21.7	21.6	21.2
Chlorine chloride	0.1	0.1	0.1	0.1
Sodium bicarbonate	0.1	0.1	0.1	0.1
Vegetable oil	2.0	1.2	1.2	1.2
Limestone	1.3	1.3	1.3	1.3
Dicalcium phosphate	1.7	1.7	1.7	1.7
Salt	0.3	0.3	0.3	0.3
Lysine	0.01	0.09	0.08	0.07
Methionine	0.19	0.17	0.15	0.2
Titanium oxide	0.5	0.5	0.5	0.5
Vitamin mineral premix[2]	0.2	0.2	0.2	0.2
Total	100	100	100	100
Nutrient composition (g/kg)				
ME poultry (MJ/kg)	12.7	12.8	12.8	12.8
Crude protein	210.1	211.0	211.0	211.2
Crude fat	59.7	58.0	58.0	58.1
Lysine	11.4	11.8	12.6	11.4
Methionine	5.4	5.2	5.0	5.4
Arginine	12.5	12.9	12.1	12.3
Methionine + cystine	6.9	6.8	6.8	6.8
Histidine	4.1	3.9	3.8	4.0
Threonine	8.5	8.2	8.2	8.2
Calcium	10.0	9.9	9.9	9.9
Available phosphorus	4.2	4.2	4.2	4.2
Sodium	1.8	1.9	2.0	1.8
Choline	1.5	1.4	1.6	1.5

[1]Diets 2, 4, 6 and 8 corresponded to diets 1, 3, 5 and 7, respectively but contained Avizyme ^{TM} 1502; 0.5g/kg and Phyzyme XP; 0.1g/kg feed, Danisco Animal Nutrition, UK.; [2]Supplied per kg of diet (mg): vitamin A (as *all-trans* retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-calcium pantothenate, 12 mg; folic acid, 2 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg, and Mo, 1 mg.

On days 7 and 21, two birds and three birds, respectively from each cage, randomly selected, were weighed and killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and removed. The contents of the ileum and caeca were collected in labelled plastic containers for the determination of nutrient digestibility and short-chain fatty acids (SCFA). Around 1 g of ileal and caecal samples were also collected separately into prepared McCartney bottles containing anaerobic

broth (see Appendix 1) for the enumeration of microbial populations. The McCartney bottles and plastic containers containing digesta samples were kept at –20 °C until bacterial enumeration and analysis of SCFA. For the determination of the TiO_2 as well as nutrient digestibility, the digesta from the ileum were pooled on a cage basis, homogenized and stored at –20 °C. Later, the samples were freeze-dried, ground through a small coffee grinding machine and stored in airtight containers at –4 °C for the chemical analysis of TiO_2, gross energy, starch and protein.

7.2.2 Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No.: AEC 09/004). Health and animal husbandry practices complied with the *Code of Practice for the Use of Animals for Scientific Purposes* issued by the Australian Bureau of Animal Health (National Health and Medical Research Council, 1990).

7.2.3 Measurements and analyses

7.2.3.1 Gross responses

Feed intake (FI), Live weight (LW), FCR and mortality were assessed as described in Section 4.2.3.1.

7.2.3.2 Organ weight

The weights of the small intestine, proventriculus plus gizzard with contents, liver, pancreas, spleen, and bursa of Fabricius were recorded on days 7 and 21 as was the body weight of the bird from which they were excised as described in Section 4.2.3.2.

7.2.3.3 Tissue protein and enzyme activities

To examine the digestive enzyme activities and protein content, the jejunal tissue was homogenized according to the method described previously by Shirazi-Beechey *et al.* (1991). As for the pancreas, the entire tissue was homogenized through a similar process to that used to homogenize the jejunal mucosa except that Milli-Q water

(Millipore Australia, North Ryde, Australia) was used instead of a phosphate buffer. Details of these methodologies were provided in Section 5.2.3.4.

7.2.3.4 Measurement of organic acids

The analytical method described by Jensen *et al.* (1995) was adopted, with modifications, for the analysis of organic acid (SCFAs, lactic acid and succinic acid) concentrations. Briefly, frozen ileal samples were thawed and homogenized by vigorous shaking. About 1.5 g (wet weight) of the homogenized digesta samples were weighed and 1 mL internal standard (0.01 M ethylbutyric acid) was added and thoroughly mixed with a vortex mixer, followed by centrifugation at 1280 × g at 5 °C for 20 minutes in a Beckman model J2-21M induction drive centrifuge with a JA-21 rotor. After that, approximately 1 mL of the supernatant was removed and thoroughly mixed with 0.5 mL of concentrated HCl and 2 mL of ether using a vortex mixer. An internal standard solution and a blank were also prepared in the same manner by replacing the supernatant with 1 mL of the standard acid mixture and 1 mL of water, respectively. Again, the mixture was centrifuged at 1640 × g at 5 °C for 15 minutes and 400 µL of the supernatant was transferred to a gas chromatograph vial (2 mL) and mixed with 40 µL of *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA). Before running on a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA), sample vials were kept in a heating block at 80 °C for 20 min and left at room temperature for 48 h. Total organic acid concentration was derived as the sum of all the organic acids observed in a sample, expressed as mg/g digesta after $\log_{10}$+1 transformation.

7.2.3.5 Titanium dioxide contents

The TiO_2 content of the ileal digesta and diet samples were measured according to the method of Short *et al*. (1996) as described in Section 5.2.3.5.

7.2.3.6 Digestibility coefficient of nutrients

The concentrations of the TiO_2 marker and of nutrients in the feed and ileal digesta were used to calculate the digestibility coefficient of protein, gross energy and starch. Diets

and ileal digesta were analyzed for protein, gross energy and starch as described in Sections 3.2.3.1 and 3.2.3.3.

The digestibility coefficient of nutrients was calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{Digesta nutrient (g/kg DM) / Digesta } TiO_2 \text{ (g/kg DM)}}{\text{Diet nutrient (g/kg DM) / Diet } TiO_2 \text{ (g/kg DM)}}$$

7.2.3.7 Enumeration of gut microbial community

Frozen ileal and caecal samples were thawed and mixed by vigorous shaking. The suspension was then homogenized for 2 min in plastic bags flushed with CO_2 using a bag mixer (Interscience, St. Norm, France) and then serially diluted in 10-fold increments in anaerobic broth according to the procedures of Engberg *et al.*(2004) and Miller and Wolin (1974), as described in Section 4.2.3.7.

7.2.4 Statistical analysis

The data were analysed using the multiple regression options of SPSS, Version 17.0.0 (SPSS Inc, 2009) for the main effects of increasing drying temperature and enzyme supplementation (Morris, 1998). The data were also subjected to GLM analysis and reported where there was an interaction between drying temperature and microbial enzyme supplementation. Data for organic acid concentrations were log-transformed ($\log_{10}$ +1) prior to analyses. Separation of means within a significant effect was conducted using Duncan's Multiple Range Test (DMRT) through post hoc procedure of SPSS. Differences between mean values were considered significant at $P \leq 0.05$, unless otherwise specified.

7.3 RESULTS

7.3.1 Gross responses

The gross response of chickens fed with the experimental diets is shown in Table 7.2. Up to 7 days of age, there was no effect of grain drying temperature and enzyme

supplementation on feed intake. However, the FI was marginally higher (P<0.078) in diets with enzyme than in unsupplemented diets (127.0 vs 122.9 g/bird). Live weight at this age was affected (P<0.011, R^2 = 0.25) by drying temperature but the trend was dependent on microbial enzyme supplementation. The microbial enzyme also improved (P<0.006, R^2 = 0.25) LW only on the diets containing sun-dried grains. The FCR was significantly improved (P<0.028, R^2 = 0.18) by increase in grain drying temperature up to 90 °C in diets. In general, FCR up to 7 d of age was better (P<0.034) on enzyme-supplemented diets than on diets without the microbial enzyme (1.56 vs 1.64 g:g).

Table 7.2 Feed intake (FI), live weight (LW) and FCR of broiler chickens at 7 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzymes[1]

Treatments				
Drying temp.	**Enzyme**	**FI (g/bird)**	**LW (g/bird)**	**FCR (g:g)**
Sun drying	–	125.5	115.9^{cd}	1.67
	+	130.2	130.4^{a}	1.45
80 °C	–	125.7	119.0^{bc}	1.59
	+	120.9	116.2^{bcd}	1.60
90 °C	–	123.3	118.6^{bc}	1.58
	+	130.9	125.8^{ab}	1.53
100 °C	–	117.0	107.7^{d}	1.73
	+	126.2	116.5^{bcd}	1.66
Pooled SEM		*1.230*	*1.370*	*0.020*
Model P		*0.078*	*<0.001*	*<0.009*
Source of variation				
Drying temperature		*NS*	*<0.011*	*<0.028*
Enzyme		*0.088*	*<0.006*	*<0.034*

[1]Each value represents the mean of 6 replicates; [a, b, c, d] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

Feed intake up to day 21 declined (P<0.001, R^2 = 0.39) with an increase in grain drying temperature of grains whereas supplementation with the microbial enzymes increased (P<0.001, R^2 = 0.39) FI but this was significant only on the diets based on sun-dried maize and the maize oven-dried at 100 °C (Table 7.3). The interaction between drying temperature and enzyme also had a significant effect (P<0.017) on FI. In general, enzyme supplementation resulted in higher FI compared to non-enzyme diets (881.1 vs 817.2 g/bird). At 21 d of age, there was a reduction (P<0.001, R2 = 0.49) in LW with increase in grain drying temperature while supplementation with microbial enzymes

(P<0.001, R2 = 0.49) improved LW only on the diets containing sun-dried grains and grains dried at 90 °C. Generally, LW was higher (P<0.001) in chickens on the enzyme-supplemented diets than on diets without enzyme (638.3 vs 547.2 g/bird). Furthermore, the interaction between drying temperature and enzyme on LW was significant (P<0.027). The FCR at this age declined (P<0.019, R^2 = 0.37) with increase in grain drying temperature while the FCR was improved (P<0.001, R^2 = 0.37) on diets supplemented with enzyme. Feed conversion ratio was also better (P<0.001) with the enzyme than without the enzyme (1.48 vs 1.62 g:g).

Table 7.3 Feed intake (FI), live weight (LW) and FCR of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzymes[1]

Treatments				
Drying temp.	**Enzyme**	**FI (g/bird)**	**LW (g/bird)**	**FCR (g:g)**
Sun drying	–	832.4^{b}	557.2^{cde}	1.61
	+	966.6^{a}	731.3^{a}	1.40
80 °C	–	841.9^{b}	546.7^{de}	1.66
	+	844.3^{b}	619.7^{bc}	1.46
90 °C	–	831.2^{b}	579.8^{bcd}	1.55
	+	863.1^{b}	634.7^{b}	1.45
100 °C	–	763.3^{c}	505.1^{e}	1.66
	+	850.4^{b}	567.5^{cde}	1.62
Pooled SEM		*10.32*	*11.76*	*0.02*
Model P		*<0.001*	*<0.001*	*<0.003*
Source of variation				
Drying temperature		*<0.001*	*<0.001*	*<0.019*
Enzyme		*<0.001*	*<0.001*	*<0.001*
Drying temp. × Enzyme		*<0.017*	*<0.027*	*<0.066*

[1] Each value represents the mean of 6 replicates; $^{a, b, c, d, e}$ Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

7.3.2 Visceral organ weight

At day 7, the relative weight of the small intestine was not significantly affected by increase in grain drying temperature but the relative weight of small intestine was decreased (P<0.046, R^2 = 0.11) in chickens on diets supplemented with microbial enzymes (Table 7.4). The relative weights of the proventriculus plus gizzard, pancreas, liver, spleen and bursa of Fabricius were not significantly affected by increase in grain drying temperature with or without enzyme supplementation.

Table 7.4 Relative weight of visceral organs (g/100 g of body weight) of broiler chickens at 7 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzymes[1]

Treatments							
Drying Temp.	**Enzyme**	**Small intest.[2]**	**Proven.+ Gizzard[2]**	**Pan-creas**	**Liver**	**Spleen**	**Bursa**
Sun drying	–	11.4	9.0	0.40	4.5	0.08	0.15
	+	11.1	9.0	0.46	5.0	0.10	0.14
80 °C	–	10.2	8.6	0.47	5.3	0.08	0.15
	+	9.9	8.6	0.43	5.1	0.09	0.16
90 °C	–	10.6	8.3	0.44	5.1	0.10	0.18
	+	9.9	8.8	0.47	5.3	0.09	0.15
100 °C	–	11.2	9.0	0.47	4.9	1.66	0.15
	+	10.0	8.7	0.48	5.3	0.09	0.14
Pooled SEM		*0.166*	*0.125*	*0.009*	*0.121*	*0.197*	*0.006*
Model P		*<0.09*	*NS*	*NS*	*NS*	*NS*	*NS*
Source of variation							
Drying temp.		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Enzyme		*<0.04*	*NS*	*NS*	*NS*	*NS*	*NS*

[1] Each value represents the mean of 6 replicates; [2] Organs were weighed with contents; NS = Non-significant; SEM = Standard error of mean.

At day 21, there was a significant increase ($P<0.04$, $R^2 = 0.10$) in the relative weight of the small intestine with increase in grain drying temperature while there was no change in the relative weight of the small intestine due to supplementation with the microbial enzymes (Table 7.5). There was no significant effect of drying temperature, enzyme or interaction between the factors on the relative weight of the proventriculus plus gizzard. The relative weight of the pancreas was not affected by grain drying temperature whereas the relative weight of the organ was decreased ($P<0.001$, $R^2 = 0.24$) in chickens on diets that were supplemented with enzymes.

At this age, the relative weight of the liver was significantly increased ($P<0.010$, $R^2 = 0.14$) with increase in grain drying temperature but this effect was absent in chickens on diets with the microbial enzymes. There was no significant effect of grain drying temperature, enzyme supplements or their interaction on the relative weight of the spleen and bursa at 21 days of age.

Table 7.5 Relative weight of visceral organs (g/100 g of body weight) of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzymes[1]

Treatment							
Drying Temp.	**Enzyme**	**Small intest[2]**	**Proven+ Gizzard[2]**	**Pan-creas**	**Liver**	**Spleen**	**Bursa**
Sun drying	–	6.0	4.6	0.37	2.8	0.09	0.20
	+	5.8	4.6	0.30	2.8	0.08	0.18
80 °C	–	6.1	4.3	0.41	3.0	0.08	0.21
	+	6.1	4.5	0.32	3.0	0.08	0.19
90 °C	–	6.5	4.5	0.45	3.1	0.10	0.16
	+	6.0	4.4	0.32	3.1	0.09	0.22
100 °C	–	6.2	4.5	0.36	3.1	0.09	0.20
	+	6.4	4.8	0.36	3.0	0.08	0.20
Pooled SEM		*0.07*	*0.07*	*0.01*	*0.04*	*0.00*	*0.01*
Model P		*NS*	*NS*	*<0.006*	*NS*	*NS*	*NS*
Source of variation							
Drying temp.		*<0.04*	*NS*	*NS*	*<0.010*	*NS*	*NS*
Enzyme		*NS*	*NS*	*<0.001*	*NS*	*0.090*	*NS*

[1]Each value represents the mean of 6 replicates; [2]Organs were weighed with contents; NS = Non-significant; SEM = Standard error of mean.

7.3.3 Tissue protein content and activity of digestive enzymes

Pancreatic tissue protein content and chymotrypsin amidase activity were not affected by grain drying temperature or enzyme supplementation at 7 days of age (Table 7.6). However, there was a significant effect ($P<0.02$) of interaction between drying temperature and enzyme on the activity of chymotrypsin amidase.

In the jejunum, there was no significant variation due to increase in grain drying temperature, enzyme or interaction between these factors on tissue protein content at day 7 of broiler chickens (Table 7.6). The activity of alkaline phosphatase was significantly increased ($P<0.03$, $R^2 = 0.16$) with increase in grain drying temperature while supplementation with microbial enzymes also marginally increased ($P<0.08$, $R^2 = 0.16$) the activity of this enzyme. The activity of maltase was significantly increased ($P<0.01$, $R^2 = 0.18$) with increase in grain drying temperature but this effect was absent on diets supplemented with microbial enzymes. The activity of sucrase also increased ($P<0.02$, $R^2 = 0.15$) with increase in grain drying temperature but this activity was not affected by the microbial enzyme supplements.

Table 7.6 Tissue protein content and activities of digestive enzymes in the pancreas and jejunum of broiler chickens at 7 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzymes[1]

Treatments		Pancreas		Jejunum			
Drying Temp.	Enzyme	Protein (mg/g tissue)	CA[2]	Protein (mg/g tissue)	AP[3]	Maltase (ηmol/mg protein)	Sucrase
Sun drying	–	259.8	1.4^{b}	283.6	3.1	174.1^{bc}	13.0^{b}
	+	312.0	2.6^{a}	310.8	2.8	151.1^{c}	11.2^{b}
80 °C	–	260.8	2.0^{ab}	349.2	2.7	216.0^{abc}	17.7^{ab}
	+	251.6	2.3^{ab}	329.2	3.0	273.9^{a}	22.4^{a}
90 °C	–	299.3	1.8^{ab}	307.0	3.0	216.7^{abc}	16.5^{ab}
	+	266.7	1.9^{ab}	311.6	3.5	236.3^{ab}	17.7^{ab}
100 °C	–	263.9	1.9^{ab}	310.0	3.0	211.6^{abc}	16.7^{ab}
	+	242.1	2.2^{ab}	277.9	4.9	277.2^{a}	22.5^{a}
Pooled SEM		*8.85*	*0.001*	*8.79*	*0.18*	*10.02*	*0.95*
Model P		*NS*	*NS*	*NS*	*0.09*	*<0.014*	*<0.021*
Source of variation							
Drying temp.		*NS*	*NS*	*NS*	*<0.03*	*<0.01*	*<0.02*
Enzyme		*NS*	*NS*	*NS*	*<0.08*	*NS*	*NS*
Drying temp. × Enzyme		*NS*	*<0.02*	*NS*	*NS*	*NS*	*NS*

[a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); [1] Each value represents the mean of 6 replicates; [2] CA; Chymotrypsin amidase (ηmol/mg protein), [3]AP; Alkaline phosphatase (µmol/mg protein); NS = Non-significant; SEM = Standard error of mean.

At day 21, the pancreatic protein content, chymotrypsin amidase activity as well as jejunal protein content and alkaline phosphatase activity were not significantly affected by increase in grain drying temperature, enzyme supplementation or interaction between the main factors (Table 7.7).

The activity of maltase rose (P<0.001, R^2 = 0.27) in line with increase in grain drying temperature but was not affected by the microbial enzyme supplements. In general, the maltase activity was higher (P<0.001) in chickens fed diets based on grain which was dried at 100 °C (281.3 ηmol/mg protein) than for diets based on sun-dried grain (224.7 ηmol/mg protein). There was an increase (P<0.007, R^2 = 0.18) in the activity of sucrase with increase in drying temperature of grains but this activity was not affected by enzyme supplementation.

Table 7.7 Tissue protein content and activities of digestive enzymes in the pancreas and jejunum of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzyme[1]

Treatments		Pancreas		Jejunum			
Drying Temp.	Enzyme	Protein (mg/g tissue)	CA[1]	Protein (mg/g tissue)	AP[2]	Maltase (ηmol/mg	Sucrase protein)
Sun drying	–	249.9	1.6	266.1	6.5	219.1^{c}	15.2
	+	252.0	1.5	273.1	6.6	230.3^{bc}	18.4
80 °C	–	273.6	1.5	277.6	6.3	248.6^{abc}	17.2
	+	283.0	1.5	290.1	5.4	238.1^{abc}	18.6
90 °C	–	257.1	1.6	268.8	4.3	275.9^{ab}	19.9
	+	269.3	1.4	267.3	4.7	247.5^{abc}	20.6
100 °C	–	251.6	1.9	250.6	6.2	283.1^{a}	20.2
	+	280.0	1.5	279.7	5.6	279.6^{ab}	22.4
Pooled SEM		*6.55*	*1.00*	*4.31*	*0.24*	*6.04*	*0.68*
Model P		*NS*	*NS*	*NS*	*NS*	*<0.037*	*NS*
Source of variation							
Drying temp.		*NS*	*NS*	*NS*	*NS*	*<0.001*	*0.007*
Enzyme		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

[a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); [1]Each value represents the mean of 6 replicates; [2]CA, Chymotripsin amidase (ηmol/mg protein); [3]AP, Alkaline Phosphatase (µmol/mg protein); NS = Non-significant; SEM = Standard error of mean.

7.3.4 Digestibility of nutrients

At 21 days of age, the ileal digestibility of protein, gross energy and starch was not significantly changed by increase in grain drying temperature and enzyme supplementation of diets (Table 7.8). However, the digestibility of protein was affected by the interaction (P<0.04) between drying temperature and enzyme, in which the digestibility of protein was higher on the diet containing grain dried at 100 °C with enzyme, than on diet containing grain dried at 90 °C without enzyme. In general, there was an increasing trend of energy digestibility in diets containing the microbial enzyme supplements.

Table 7.8 Ileal digestibility of protein, gross energy and starch of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzymes[1]

Treatment				
Drying temp.	**Enzyme**	**Protein**	**Gross energy**	**Starch**
Sun drying	–	0.83^{abc}	0.76	0.98
	+	0.85^{ab}	0.78	0.98
80 °C	–	0.85^{ab}	0.77	0.98
	+	0.81^{bc}	0.73	0.96
90 °C	–	0.80^{c}	0.75	0.96
	+	0.83^{abc}	0.77	0.97
100 °C	–	0.83^{abc}	0.75	0.97
	+	0.86^{a}	0.78	0.98
Pooled SEM		*0.005*	*0.007*	*0.002*
Model P		*0.076*	*NS*	*<0.05*
Source of variation				
Drying temp		*NS*	*NS*	*NS*
Enzyme		*NS*	*NS*	*NS*
Drying temp. × Enzyme		*<0.04*	*NS*	*NS*

[1]Each value represents the mean of 6 replicates; [a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

7.3.5 Organic acids

The concentrations of organic acids in the ileum of broiler chickens at day 21 are shown in Table 7.9. Formic acid content increased (P<0.001, R^2 = 0.25) with increase in drying temperature of grains used in the diets but the concentration of formic acid was not changed by supplementation with microbial enzymes. There was a significant increase (P<0.001, R^2 = 0.34) in the concentration of acetic acid with increase in grain drying temperature but this was not affected by inclusion of microbial enzymes in diets. The concentration of lactic acid was not significantly affected by drying temperature of grains with or without enzyme supplementation or interaction between them at day 21.

Table 7.9 Concentrations of various organic acids (mg/g digesta) in ileal content of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzyme[1]

Treatments		Ileum		
Drying temp.	**Enzyme**	**Formic acid**	**Acetic acid**	**Lactic acid**
Sun drying	–	0.15	0.41	0.77
	+	0.23	0.43	0.79
80 °C	–	0.25	0.42	0.88
	+	0.23	0.44	0.83
90 °C	–	0.33	0.51	0.93
	+	0.30	0.51	0.92
100 °C	–	0.35	0.57	0.86
	+	0.32	0.60	0.59
Pooled SEM		*0.02*	*0.02*	*0.05*
Model P		*<0.03*	*<0.01*	*NS*
Source of variation				
Drying temp.		*<0.001*	*<0.001*	*NS*
Enzyme		*NS*	*NS*	*NS*

[1]Each value represents the mean of 6 replicates; Data were transformed (log_{10} + 1) prior to analysis; NS = Non-significant; SEM = Standard error of mean.

The concentrations of formic and acetic acids in the caecal contents were not changed in response to increase in drying temperature of grains used in the diets with or without enzyme supplementation (Table 7.10). The concentration of propionic acid increased ($P<0.03$, $R^2 = 0.12$) with increase in grain drying temperature but enzyme supplementation had no effect. The concentrations of isobutyric acid, butyric acid, and isovaleric acids were not affected by increase in drying temperature of grains, enzyme supplementation or interaction between the main factors. The concentration of valeric acid increased ($P<0.018$, $R^2 = 0.12$) as grain drying temperature increased but the concentration of this acid was not changed as a result of supplementation with microbial enzymes. There was a significant reduction in the concentration of lactic acid ($P<0.02$, $R^2 = 0.14$) and succinic acid ($P<0.008$, $R^2 = 0.15$) in birds on diets with the enzyme supplementation but the concentration of these acids was not affected by grain drying temperatures.

Table 7.10 Concentration of various organic acids (mg/g digesta) in caecal content of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzyme[1]

Treatments		Caecum								
Drying temp.	**Enzyme**	Formic	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Lactic	Succinic
Sun drying	–	0.16	1.7	0.73	0.28	0.81	0.12	0.26	0.48	1.04
	+	0.12	1.8	0.80	0.29	0.83	0.10	0.25	0.16	0.67
80 °C	–	0.13	1.8	0.78	0.30	0.78	0.13	0.27	0.09	0.72
	+	0.12	1.8	0.79	0.29	0.87	0.11	0.28	0.15	0.80
90 °C	–	0.13	1.8	0.85	0.31	0.87	0.12	0.31	0.38	0.87
	+	0.14	1.8	0.86	0.31	0.81	0.12	0.28	0.14	0.64
100 °C	–	0.16	1.8	0.82	0.26	0.83	0.09	0.29	0.19	1.02
	+	0.15	1.8	0.91	0.31	0.82	0.11	0.30	0.11	0.70
Pooled SEM		*0.01*	*0.0*	*0.02*	*0.01*	*0.01*	*0.01*	*0.01*	*0.03*	*0.04*
Model P		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.013*	*<0.05*
Source of variations										
Drying temp.		*NS*	*NS*	*<0.03*	*NS*	*NS*	*NS*	*<0.018*	*NS*	*NS*
Enzyme		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.022*	*<0.008*

[1]Each value represents the mean of 6 replicates; Data were transformed (log_{10}+1) prior to analysis; NS = Non-significant; SEM = Standard error of mean.

7.3.6 Gut microflora

In the ileal content, there was no significant effect of grain drying temperature or enzyme supplementation on total anaerobic bacterial count (Table 7.11). However, the interaction between drying temperature and enzyme supplementation on this bacterial count was significant ($P<0.020$). The populations of lactic acid bacteria ($P<0.054$, $R^2 = 0.10$) and lactobacilli bacteria ($P<0.001$, $R^2 = 0.28$) were decreased on diets with enzyme supplementation but these were not affected by increase in grain drying temperature. There were no significant changes in the populations of Enterobacteria and *C. perfringens* in the ileal content at 21 days of age as a result of variation in grain drying temperature, enzymes or their interaction.

In the caecal content, the total anaerobic bacterial count was not significantly changed by increase in drying temperature of grains but the population of these species was increased ($P<0.010$, $R^2 = 0.17$) on diets that were supplemented with microbial enzymes (Table 7.11). The population of lactic acid bacteria was increased ($P<0.010$, $R^2 = 0.18$) as a result of increase in grain drying temperature but this effect was absent on diets with microbial enzymes. There were no significant changes in the populations of lactobacilli, Enterobacteria and *C. perfringens* in the caecal content at 21 days of age as a result of variation in grain drying temperature, enzyme supplementation or their interaction.

Table 7.11 Bacterial counts (log_{10} cfu /g digesta*)* in ileum and caeca of broiler chickens at 21 days of age on the different diets based on sun-dried maize or artificially dried at different temperature with or without enzyme[1]

Treatments		Ileal content					Caecal content				
Drying temp.	**Enzyme**	**Anaero-bic**	**Lactic acid**	**Lacto-bacilli**	**Entero bacteria**	***C. perfrin gens***	**Anaero-bic**	**Lactic acid**	**Lacto-bacilli**	**Entero bacteria**	***C. perfrin gens***
Sun drying	–	7.4^{a}	7.6	7.4	4.3	5.5	7.9	8.3	8.1	7.6	6.4^{c}
	+	7.0^{abc}	8.0	8.0	4.7	6.0	8.1	8.4	8.2	8.1	7.1^{a}
80 °C	–	6.7^{bc}	7.9	7.6	4.8	5.8	7.9	8.3	8.0	7.7	6.9^{ab}
	+	6.5^{c}	7.5	7.2	5.0	5.5	8.2	8.6	8.2	7.9	6.6^{bc}
90 °C	–	7.3^{a}	8.0	7.8	4.6	6.1	8.2	8.6	8.3	8.0	6.9^{abc}
	+	6.9^{abc}	7.9	7.4	5.2	6.4	7.7	8.6	8.2	7.7	7.0^{ab}
100 °C	–	6.5^{c}	7.9	7.5	4.7	5.9	7.8	8.7	8.2	7.7	7.1^{a}
	+	7.1^{bc}	7.7	7.2	4.6	5.9	8.1	8.7	8.0	8.0	6.8^{abc}
Pooled SEM		*0.07*	*0.07*	*0.07*	*0.08*	*0.09*	*0.06*	*0.05*	*0.04*	*0.06*	*0.06*
Model P		*<0.002*	*NS*	*<0.023*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.036*
Source of variation											
Drying temp.		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.010*	*NS*	*NS*	*NS*
Enzyme		*NS*	*0.054*	*<0.001*	*NS*	*NS*	*<0.010*	*NS*	*NS*	*0.092*	*NS*
Drying temp.×Enzyme		*<0.02*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.011*

[1]Each value represents the mean of 5 replicates for each treatment group; [a, b, c] Values with unlike superscripts within each column are significantly different (P<0.05); NS = Non-significant; SEM = Standard error of mean.

7.4 DISCUSSION

7.4.1 Gross responses

The present study demonstrated that the maize dried either naturally under the sun or artificially dried at 90 °C adequately supported chicken performance, including FI, LW and FCR, up to 21 days of age. Enzyme supplementation also improved digestibility and bird performance on diets based on these two batches. Therefore, sun drying, as practised in many areas is a suitable method for processing of maize, which will be used as broiler feed. However, where sun drying is not possible due to climatic or other factors, artificial drying at 90 °C is recommended, although enzyme supplementation may be needed to get the best results. This improvement is, perhaps, due to better chemical composition (Bhuiyan *et al.*, 2010) and ultra-structural properties of grain dried at 90 °C or sun-dried grain compared to grain dried at other high temperatures (Chapter 6). Previous researchers, for example, Kaczmarek *et al.* (2007) reported a somewhat similar finding that is, that high drying temperatures possibly have an adverse effect on the nutritional value of maize used for broiler feeding, and that exogenous enzymes are effective in partially ameliorating this inconsistency in caloric value if the grain is dried at more than 100 °C. During the whole experimental period, the worst performance results (LW and FCR) were found in the grain batch dried at 100 °C. This may be due to the decrease of its nutritional quality through high temperature drying, as reported previously (Brown, 1996; Iji *et al.*, 2003).

The improvement in body weight through microbial enzyme supplementation of the diet based on 90 °C-dried maize is consistent with our previous findings that maize dried at this temperature has higher amylose concentration (Chapter 6). Enhancement of performance in broiler chickens, turkey and meat type ducks by microbial enzyme supplement was reported previously (Cafe *et al.*, 2002; Hong *et al.*, 2002; Troche *et al.*, 2007). In addition, Zanella *et al.* (1999) showed that enzyme supplementation improved body weight and feed conversion ratio by 1.9 and 2.2 %, respectively in broiler chickens.

7.4.2 Visceral organ weight

In the present study, the relative weight of the small intestine was affected by drying temperature of the grain used in the diet and by supplementation with enzymes. The relative weight of the small intestine of chickens fed diets containing sun-dried grain and without enzyme supplementation was found to be higher than that of birds on diets containing grain artificially dried at other temperatures, and with enzyme supplementation. These increases in the mass of the small intestine are approximately proportional to the increase in body weight, and might, therefore, be a general consequence of faster growth. The increase may be due to better quality of the sun-dried grains, in particular the better starch and protein quality. However, Iji *et al.* (2003) did not find any significant variation in the development of the GI tract, including the small intestine in birds fed on diets containing maize oven-dried at different temperatures. This contrast in results is perhaps due to the fact they tested low moisture grains, which would cause less retrograding of the starch. The weights of other organs such as the liver and pancreas were also affected by drying temperature and enzyme supplementation. This is most probably due to the quality of grain; in particular the high proportion of amylose when grain is oven-dried (Bhuiyan *et al.*, 2010) and it may be that the microbial enzyme further aided the digestion of the carbohydrases.

7.4.3 Tissue protein content and activity of digestive enzymes

In this study, significant differences were observed in pancreatic protease (chymotrypsin amidase) activity due to interaction between drying temperature and enzyme, and higher pancreatic protease activity was found in birds on diets containing sun-dried grains with enzyme supplementation than was found in groups on unsupplemented diets. This may be due to the better quality of starch and microbial enzyme supplementation, with the microbial enzyme perhaps creating a favourable environment for the secretion of endogenous amylolytic enzyme. However, other pancreatic enzyme activities and protein concentration were not affected by the grains dried at different temperatures and microbial enzyme supplements. A dissimilar finding was reported by Mahagna *et al.* (1995), who observed a reduction in the activity of

pancreatic enzymes *in situ* and in intestinal digesta as a result of supplementation with a microbial enzyme with proteolytic and amylolytic activities.

The relationship between high-moisture grain dried at different temperatures and the activity of intestinal digestive enzymes has apparently not been previously examined. A reduction in jejunal tissue protein content was observed in this study at d7, due to microbial enzyme application. Similar results have been reported by other workers (Danicke *et al.*, 2000a; Iji *et al.*, 2004) although using grain of lower moisture content. The activity of jejunal mucosal disaccharidases (maltase and surcease) was significantly affected by drying temperature of grains but not by supplemental enzyme, and higher maltase and sucrase activity was observed in birds on diets with grain dried 80, 90 and 100 °C compared to sun-dried grain. The increase in intestinal enzymes is stimulated mechanically by chyme passing through the digestive tract (Duke, 1986). This may be due to DM content of diets. For example, based on DM, the diets containing grains dried at 80, 90 and 100 °C were higher in starch than the sun-dried grain diet. The high activity of maltase and sucrase in the birds receiving these diets is probably related to substrate (maltose and sucrose) availability in the jejunum.

7.4.4 Nutrient digestibility

In this study, there was a significant effect of interaction between drying temperature and enzyme on protein digestibility at day 21. Protein digestibility was higher on diets with 100 °C-dried grains, with enzyme supplementation, than on diets with 90 °C-dried grain without enzyme supplementation. One of the test microbial enzymes, Avizyme 1502, contains a protease and may improve protein digestion. This is in agreement with the findings of Zanella *et al.* (1999), who showed that both nutrient digestibility and broiler performance were improved with the use of a mixture of enzymes such as xylanase, amylase and protease in maize/soya bean meal diets.

There was a significant effect of drying temperature on starch digestibility, with higher starch digestibility found in birds on diets with sun-dried and 100 °C-dried grain than grain dried at other temperatures. However, the enzyme supplementation also enhanced starch digestibility in each case. The starch component of maize is considered to be highly digestible but Brown (1996) reported that such starch may be resistant to

digestion, and such resistance increases when grains are dried artificially. With such starch, digestion would be completed in the hindgut and enzyme supplementation may improve digestion of this fraction. This phenomenon could warrant the use of exogenous enzymes (xylanase, β-glucanse, pectinase, cellulase, galactanase) to increase the digestibility of nutrients found in maize (Meng and Slominski, 2005).

In this study, it was found that protein and starch digestibility was enhanced in diets containing grains dried at 90 and 100 °C. This may be due to the role that the addition of microbial phytase in poultry diets plays in increasing calcium and phosphorus availability, a finding that is well established in the literature. Phytic acid is a critical factor present in feedstuffs and it binds minerals, proteins, lipids and starch (Thompson and Yoon, 1984), reducing the digestibility of these nutrients for poultry (Sebastiana *et al.*, 1997). Recent studies have also shown a beneficial effect of phytase addition to broiler diets on metabolizable energy and total amino acid digestibility (Namkung and Leeson, 1999; Ravindran *et al.*, 1999). This also may be due to the activity of exogenous enzymes that improve the nutritive value of maize by hydrolyzing polysaccharides that are involved in the encapsulation of starch or protein, thereby rendering compounds available for digestion that were previously not accessible to endogenous enzyme (Bedford, 1996). It may possibly be that the use of various exogenous enzymes can improve the nutritional value of diets by reducing the loss of endogenous material (Selle *et al.*, 2000; Danicke *et al.*, 2000b; Cowieson *et al.*, 2003). This may be due to the variation in chemical composition among different diets due to drying temperature. For example, Barteczko *et al*. (2008) speculated that nutrient and energy digestibility of maize grain depended on its chemical composition and that a higher nutrient content leads to increased digestibility.

7.4.5 Ileal and caecal microflora and microbial activity

There was an increase in the number of total anaerobic bacteria, particularly in the ileum due to drying temperature as well as the interaction between enzyme and drying temperature. A higher anaerobic count was found in birds on diets based on sun-dried, compared to 80 °C-dried grain, but there was no significant difference between diets based on grain dried at 90 °C and those based on grain dried at 100 °C. It is likely that

these variations are due to a decrease in the quality of the grain. Although the ileal viscosity of birds was not measured in the present study, it is possible that there were some negative effects due to oven drying. However, *in vitro* viscosity has been measured using oven-dried grain and no significant variation was found (Bhuiyan *et al.*, 2010). There was a significant effect of enzyme supplementation in the number of lactobacilli with a higher lactobacilli population found in the diet without enzyme supplementation but only to a minor extent. Carbohydrates may be digested on diets without microbial enzymes. Such carbohydrates are fermented in the hind gut, leading to an increase in microbial populations. The populations of other species such as lactic acid bacteria, enterobacteria and *C. perfringens* were not affected by grain quality or enzyme supplementation, although the count of *C. perfringens,* the causative agent of necrotic enteritis, was slightly increased.

In caecal content, there was a significant variation in total anaerobic count with higher total anaerobic bacteria found in chickens on the enzyme-supplemented diets than those on the unsupplemented diet. This is an unusual occurrence as microbial enzymes tend to increase ileal digestion of nutrients and reduce caecal fermentation (Apajalahti and Bedford, 1999; Fernandez *et al.*, 2000; Apajalahti *et al.*, 2004). On the other hand, there was a significant increase in the population of *C. perfringens,* associated with diets based on sun-dried and 100 °C-dried grain with enzyme supplementation, than sun-dried grain diets without enzyme supplementation. However, there was no sign of a necrotic enteritis problem in this experiment.

In the current study, there was a significant effect of drying temperature on the concentration of formic and acetic acids in the ileum of broiler chickens, and the higher concentration of formic acid was associated with grain dried at 100 °C rather than sun-dried grain. This result suggests that the grains dried at 80 and 100 °C were fermented more by the ileal microflora. Therefore, the growth and activity of formic acid- and acetic acid-producing bacteria in the ileum are likely promoted by the grain dried at 80 and 100 °C. The composition of microbiota and increase in their digestive activity can have positive as well as negative effects on the host (Gabriel *et al.*, 2006). Among the positive effects are competitive exclusion, increased SCFA production and release of nutrients that can be absorbed in the intestine. Similar significant changes in the

concentration of lactic acid were found due to enzyme supplementation, with a higher lactic acid content found in birds on diets without enzyme supplementation than those on supplemented diets. Lactate was the most predominant organic acid in the ileal content, while acetic acid was predominant in the caecal digesta. Lactic acid is an electron sink, further oxidized to other SCFAs such as acetic, propionic and butyric acids in the caeca due to longer retention time of the caecal digesta (Vidanarachchi, 2006). An increased SCFA production through bacterial fermentation of un-digested carbohydrates such as resistant starch also results in lower pH, which is an accepted mechanism for the inhibition of acidophilic bacteria such as enterobacteria and *C. perfringens* in broiler chickens (Terada *et al.*, 1994; Orban *et al.*, 1997). In the current study, however, no reductions in the populations of enterobacteria or *C. perfringens* were found due to grain drying temperature or enzyme supplementation.

7.5 CONCLUSIONS

The present study showed that diets based on sun-dried maize or maize dried at 90 °C gave comparatively better performance in terms of feed intake, live weight, live weight gain and FCR of broilers than diets based on maize dried at other temperatures. Moreover, there was a significant positive response to supplementation with microbial enzymes. The relative weight of the small intestine and liver, the bacterial populations and starch digestibility were also higher in birds on diets based on sun-dried maize compared to those based on artificially dried grain.

Considering the results from this experiment, it may be concluded that, there is little or no difference in the nutritive value of sun-dried grains or grains dried artificially at 90 °C but temperatures lower or higher than 90 °C may be unsuitable. Supplementation with a suitable enzyme may also be of some benefit in terms of visceral organ development, performance, protein digestibility and gut microbial activity.

CHAPTER 8 GENERAL DISCUSSION

8.1 INTRODUCTION

As reviewed in Chapter 2, the quest for the efficient use of cereal grains demands a great knowledge of the variations in their chemical and physical characteristics, and their nutritional value due to the source, type, variety and post-harvest processing. On the other hand, growth performance, microbiology, gut physiology and immunology of birds on diets based on such grains are influenced by the nutritional quality of the grains. However, previous investigations on the nutritive quality of grains with or without any microbial enzyme supplementation have yielded mixed results with varying levels of success, both in terms of feed consumption and utilization. There are a number of important obstacles to determining the optimum use of grains. The first consideration is post-harvest processing; in particular the variation in physiochemical properties of high-moisture maize grain due to artificial drying. Secondly, subsequent variation in feed utilization and growth performance is not clearly understood. Thirdly, there has been a lack of knowledge on the interrelationship between gut microflora and exogenous enzyme cocktails on the growth of birds on such diets. Therefore, five experiments were conducted in the present work to examine the nutritive value of maize from various sources with or without heat treatment and to determine the optimal drying temperature of high-moisture maize, and particle size, to maximize the digestibility of microbial enzyme-supplemented grain.

8.2 GRAIN SOURCE

In this study, maize (sun-dried) was used from only three different sources; Moree in NSW and Emerald and Downs in Queensland of Australia. There was little variation observed in proximate composition and ultra-structure and nutritive quality between maize from the three sources (Chapter 3). The sources of maize did result in differences in LW of birds in early life and gross energy and starch digestibility at a later age (Chapter 4). This may be a result of compositional differences between the sources, and this is supported by the findings of Cowieson (2005), who reported that the chemical

composition and nutritional value of maize differs from batch to batch, resulting in conspicuous variation in its energy value for poultry. In the current study, it is obvious that the mineral contents of maize varied from one source to the other. The digestibility of energy and starch was affected by the source of maize and this being most noticeable in birds on diets based on maize from Emerald and Moree. This could be due to differences in chemical composition of grain from the different sources. Higher variation could be expected, probably, if a wider range of sources was tested.

8.3 HEAT TREATMENT/ ARTIFICIAL DRYING

In this study, there was little variation observed in proximate composition and ultrastructures**,** and nutritive quality of sun-dried maize due to heat treatment, but these variations were not observed in all cases (Chapter 3). This may, perhaps, be due to the heating of low-moisture maize. However, there was a huge variation found in the above variables when high-moisture maize was dried artificially at different temperatures (Chapter 6). In particular, the size of the starch granule is an important factor in determining the energy value of starch, with smaller granules having a relatively larger surface area and so a greater potential for hydrolysis by endogenous amylase (Carre, 2004). The magnitudes of these changes, especially the varying proportions of amylose and amylopectin, were found to be dependent upon the moisture content during heat treatment, and the grain sources.

In the present study, one of most interesting findings was that high-temperature drying lowers the content and quality of starch and also that starch content decreases significantly as the drying temperature increases above 90 °C, which was consistent with findings of others (Watson and Hirata, 1961; Brown *et al.*, 1981; Brown, 1996). Artificial drying results in the annealing of starch, while long periods of sun-drying have been known to cause stack-burn, a defect leading to downgrading of the quality of the grain (Panigrahi *et al.*, 1996). Once the thermal process is completed and the grain cools, retrogradation begins (Atwell *et al.*, 1988) and then starch returns to a more ordered state, where both amylose and amylopectin form double helical associations, the extent of which is dependent on the amylose: amylopectin ratio (Jacobson *et al.*,

1997; Klucinec and Thompson, 1999). These changes in structure associated with heating also alter the subsequent digestibility of the starch.

The present study demonstrated that the maize dried either naturally by the sun or force-dried at 90 °C adequately supported chicken performance, including FI, LW and FCR up to day 21 (Chapter 7). Enzyme supplementation also improved digestibility and birds' performance on diets containing grains dried under these two conditions. This improvement was, perhaps, due to better chemical composition (Bhuiyan *et al.*, 2010) , and ultra-structural properties found in grain dried at 90 °C and in sun-dried grain compared to grain dried at other high temperatures (Chapter 6). Therefore, sun drying, as practised in many areas, is a suitable method for the processing of maize which will be used as broiler feed. However, where sun drying is not possible due to climatic or other factors, artificial drying at 90 °C is recommended, although enzyme supplementation may be needed to get the best results. Kaczmarek *et al.* (2007) reported a somewhat similar finding, that is, that high drying temperatures possibly have an adverse effect on the nutritional value of maize used for broiler feeding, and that exogenous enzymes are effective in partially ameliorating this inconsistency in caloric value if the grain is dried at more than 100 °C. During the study on high-moisture maize, the worst performance results (LW and FCR) were found in the batch dried at 100 °C. This may be due to the decrease of its nutritional quality through high temperature drying, as reported previously (Brown, 1996; Iji *et al.*, 2003).

The relative weight of the small intestine of chickens fed diets containing sun-dried grain and without enzyme supplementation was found to be higher than that of birds on diets containing grain artificially dried at other temperatures (Chapter 7). These increases in the mass of the small intestine are approximately proportional to the increase in body weight, and might, therefore, be a general consequence of faster growth. The increase may be due to the better quality of the sun-dried grains, in particular the better starch and protein quality.

The activity of jejunal mucosal maltase and sucrase were significantly affected by drying temperature of grains but not by supplemental enzyme, and higher maltase and sucrase activity was found in birds on diets with grain dried at 80, 90 and 100 °C compared to sun-dried grain. This may be due to the high DM content of diets. In this

study, there were significant differences in relation to the main factor, that is, the drying temperature of grain in respect of alkaline phosphatase, and this may indicate more mature enterocytes in birds fed on the sun-dried and 100 °C-dried grain. The relationship between high-moisture grain dried at different temperatures and the activity of digestive enzymes has apparently not been previously examined. A reduction in jejunal tissue protein content was observed in this study at day seven, due to microbial enzyme application. Similar results have been reported by other workers (Danicke *et al.*, 2000a; Iji *et al.*, 2004), although they used grain of lower moisture content.

A higher population of anaerobic bacteria was found in birds on diets based on sun-dried, compared to 80 °C-dried grain, but there was no significant difference between diets based on grain dried at 90 °C and those based on grain dried at 100 °C. There was an increase in the number of total anaerobic bacteria, particularly in the ileum due to drying temperature as well as the interaction between enzyme and drying temperature. It is likely that these variations are due to a decrease in the quality of the grain. This thesis shows the significant variations in physiochemical composition and nutritive value of high-moisture maize grain due to post-harvest oven drying (Chapter 3, 6 and 7). In brief, sun drying is the best, however, when sun drying is not possible artificial heating at 90 °C for 24 h is recommended when maize is harvested with high moisture content.

8.4 MILLING

From this study it is clearly evident that milling technique had no effect on overall performance, but the interaction between hammer milling and particle size affected FI, LW and LWG up to d7 (Chapter 4). This may have resulted due to a higher proportion of coarse particles in hammer-milled diets compared to roller-milled diets.

In this study, there was no significant effect of milling technique on nutrient digestibility; however, particle size appears to be more important. A study by Roelof *et al.* (2001) found that milling method (hammer or roller) had no effect on the extent of starch digestion but enhanced its digestion rate, and that maximum digestibility results from roller-milled maize. The differences in starch digestibility due to maize variety

may be due to the variable starch composition of grain sources. Overall, milling technique is not important for maize grinding.

8.5 PARTICLE SIZE

In this study, feed intake was significantly affected by particle size only up to day 7, but not at 21 days of age. The particle size of maize consumed by birds has been found to be related to beak development and the age of the birds (Moran, 1982a; Portella *et al.*, 1988). In the present study, there was an increase in the relative weight of the proventriculus-gizzard and liver in early age for chickens on coarse particle diets, while the relative weight of the immune organ, the bursa, was increased on the fine particle diets at the later stage (Chapter 4). Similar results were observed by Nir *et al.* (1994c) and Svihus *et al.* (1997), who acknowledged that gizzard size of broilers became significantly bigger when they were fed diets with coarser particles than when they were fed fine mash. Gizzard development can be achieved by manipulating the feed particle size of diets and this can be employed as a nutritional strategy (Nir *et al.*, 1995; Engberg *et al.*, 2002).

Moreover, protein and energy digestibility was increased in chicks fed on fine particle diets. These findings are in contrast to the report by Parsons *et al.* (2006), who found that feeding medium to coarse particle maize improved nutrient digestion. This may, however, be due to better gizzard development and function. In this study, the higher CP and gross energy digestibility was influenced by the particle size of diet, fine particle being more beneficial than coarse particle. It may be that smaller particle size increases the surface area available to enzyme activity, allowing more nutrients to be digested and absorbed through the small intestine. This finding supports those of Cumming (1994), and Forbes and Covasa (1995), who stated that the particle size of feed may affect the digestive tract of birds and consequently nutrient digestibility as well.

In the present study, there was no significant change in bacterial populations in chickens due to grain particle size. However, the population of *C. perfringens* was affected by interactions between milling technique and particle size. This finding was

supported to some extent by the results of Engberg *et al.*(2002) who mentioned that the structural properties of feed, grain particle size and feed formulation can influence the intestinal microflora of poultry. For example, an increase in retention time in the gizzard and lower gizzard pH may not only kill ingested enteric pathogens, but also increase the fermentation by symbiotic bacteria in the crop that act as seed stock to colonize the lower digestive tract and competitively exclude pathogens (Engberg *et al.*, 2004).

In the current study, there was a significant reduction in the population of *C perfringens* in caecal content due to coarse particle size. This is expected as a well-developed gizzard is associated with improvement in gut motility (Ferket, 2000) and may prevent pathogenic bacteria such as *C. perfringens* from entering the small intestine (Bjerrum *et al.*, 2005). Such diets may reduce the risk of coccidiosis and other enteric diseases (Engberg *et al.*, 2004; Bjerrum *et al.*, 2005). In brief, particle size of grain played a role and fine (<2 mm) maize particle provided the best results.

8.6 MAIZE INCLUSION LEVEL

The results of this current study demonstrate a significant improvement in FI, LW and FCR of broiler chicks on starter diets with a higher MIL (MM and HM) (Chapter 5). The significant enlargement of the small intestine, liver and proventriculus-gizzard of birds on the higher MIL may be partly attributed to the influence of grain texture resulting in stimulation of the digestive organs. This finding is in agreement with that of Zarghi and Golian (2009), who reported an increase in the weight of the gizzard and small intestine with an increase in triticale in the diet when measured at 18 and 42 days of age.

In this study, MIL had no effect on either pancreatic and jejunal tissue protein content or mucosal digestive enzyme activities in broiler chickens (Chapter 5). The slight increase in the numbers of total anaerobic bacteria and lactobacilli in the ileum of birds fed on a high MIL diet with supplemental enzyme may suggest a positive effect of substrate availability for microbial growth especially at a higher maize level. In this study, MM (500 g/kg diet) was better with microbial enzyme, however HM (750 g/kg diet) was best without the enzyme supplement. In commercial diets maize is used at a level

comparable to the MM level tested in this study. Birds would, however, tolerate higher levels as maize is low in soluble NSP (Englyst, 1989; Choct, 1997), as demonstrated in the current study

8.7 ENZYME SUPPLEMENTATION

The addition of the enzyme supplement improved the LW and FCR in birds on the experimental diets (Chapter 5 and 7). A similar performance trial conducted by Zanella *et al.* (1999), found that enzyme supplementation of identical basal diets improved LW and the FCR by 1.9 and 2.2 %, respectively. Maize may respond to enzyme supplementation due to the presence of non-starch polysaccharides, a large part of which is arabinose and xylose (Chesson, 2001). In the current study, birds receiving a diet with a high MIL and/or supplemented with exogenous enzyme had improved growth performance throughout the experimental period. Cowieson *et al.* (2006c) articulated that exogenous xylanase, amylase, protease and phytase can be used successfully in a strategically formulated low-nutrient density diet to maintain a similar level of performance to that of birds fed on a nutritionally adequate diet.

Feeding at high inclusion of maize still supported high digestibility of CP, gross energy and starch. This supports perception that maize has a relatively high and consistent nutritional value for poultry and so there is little to be gained by adding enzyme. However, the digestibility of nutrients by broilers fed on maize-soybean meal-based diets can be improved by the use of a combination of xylanase, amylase, protease and phytase (Zanella *et al.*, 1999; Zanella *et al.*, 2004; Cowieson *et al.*, 2006c). In the present study, protein and starch digestibility was enhanced in diets containing grains dried at 90 and 100 °C (Chapter 7). The starch component of maize is considered to be highly digestible, but Brown (1996) reported that such starch may be resistant to digestion, and such resistance increases when grains are dried artificially. The enzyme mixture may have improved digestion of this fraction. The above phenomenon could warrant the use of exogenous enzymes (xylanase, β-glucanase, pectinase, cellulase, galactanase) to increase the digestibility of nutrients found in maize (Meng and Slominski, 2005).

In this study, a significant change were observed in chymotrypsin amidase activity due to interaction between drying temperature and enzyme, and higher pancreatic protease activity was found in birds on diets containing sun-dried grains with enzyme supplementation than was found in groups on unsupplemented diets. This may be due to the better quality of starch and microbial enzyme supplementation, with the microbial enzyme perhaps creating a favourable environment for the secretion of endogenous amylolytic enzyme.

Generally, bacterial populations such as total anaerobic bacteria and lactobacilli are regarded as beneficial to the host and can be increased through enzyme supplementation. Rosin *et al.* (2007) found a mixed effect on gut bacterial population; in particular the number of *E. coli* was reduced due to the inclusion of enzymes. This effect may depend on the nature of the carbohydrate source and enzyme type. It is clear that the microbial enzymes acted positively in terms of productivity and highest utilization of maize grain for broiler chickens. In particular, microbial enzymes were very effective in diets when grains were dried artificially.

8.8 CONCLUSIONS AND RECOMMENDATIONS

The chemical composition and ultra-structure of low-moisture/sun-dried maize grain from different sources were not affected by artificial heating apart from starch, CP and ME. Starch quality declined with an increase in amylose contents due to heat treatments and CP and ME increased due to an increase in DM content as a result of heating. However, there was much variation in these values due to artificial drying of high-moisture maize grain at different temperatures. In particular, there was a reduction in the concentrations of phytate-P, digestible starch, amylopectin and soluble NSP. The changes in starch composition may significantly affect the nutritive value of the grains. These effects were most pronounced at 100 °C. If it is necessary for high-moisture maize to be artificially dried, then the drying temperature should be lower than 100 °C.

The present study indicates that source, milling and particle size of grains are important in mash diets in terms of bird performance, especially in early life. In particular, fine particle size resulted in an increase in FI, and one of the maize sources (Moree)

enhanced LW at the same growth phase. However, FCR was improved on hammer-milled Downs maize in the second and third weeks of age. Large particle size has an initial stimulatory effect on the gizzard and subsequent effects on the liver and bursa. The gut environment and its community were influenced by milling technique and particles size of diets in broiler chickens.

The results showed that maize grain, at relatively high dietary levels, when supplemented with appropriate exogenous enzymes, still supported productivity to levels that are close to commercial standards. The implication of this is that it may be possible to reduce the inclusion levels of more expensive protein ingredients leads to benefits in terms of feed intake in later age, live weight and FCR as well as the development of the GIT.

It may be assumed that, for broiler chickens, there is little or no difference in the nutritive value of sun-dried grain compared to grain artificially dried at 90 °C. Supplementation with a suitable enzyme may also be of some benefit in terms of visceral organ development, performance, protein digestibility and gut microflora.

There is a need for future research to elucidate the variations in chemical composition and nutritional value for broiler chickens of high-moisture maize grains in long-term storage after drying. Wheat grain quality is known to change during storage but wheat is generally harvested at storable moisture content.

There is also a need to investigate the dose responses of microbial enzymes in diets containing maize grains dried artificially at 90 °C and higher on bird performance.

Another area worth investigation is gut histology of broiler chickens on the kinds of diets that were used in the present study.

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APPENDIX

Appendix 1 The composition of anaerobic broth (per litre)

Yeast extracts	2.5 g
Peptone from casein	2.5 g
Solution A	167 mL
Solution B	167 mL
Resazurin solution	1 mL
Hemine solution	5 mL
Tween 80	1 mL
MillQ water	add to 1 litre

Solution A (per litre)

NaCl	5.4 g
KH_2PO_4	2.7 g
$CaCl_2.H_2O$	0.16 g
$MgCl_2.6H_2O$	0.12 g
$MnSO4.\ 4H_2O$	0.07 g
$CoCl_2.6H_2O$	0.06 g
$(NH_4)_2SO4$	5.4 g
$FeSO_4.7H_2O$	0.05 g

Solution B (per litre)

$K_2HPO_4.3H_2O$	2.7 g/ litre

Resazurin solution

100 mg resazurin in water	100 mL

Hemine solution

0.05 g hemine in 0.02% NaOH	100mL