CHAPTER 1 GENERAL INTRODUCTION

1.1 BACKGROUND

Cereal grains are used primarily in animal diets as energy sources, with grains constituting about 60 to 70% of commercial poultry diets (Black *et al.*, 2005). Cereal grains such as wheat and maize have played a major role in poultry feeding for decades; however, the cost of those grains has increased sharply over the past few years owing to drought conditions in areas of wheat production and the increased use of maize for production of ethanol. To cope with those constraints, it is necessary to identify other grains that could decrease dependence on wheat and maize in poultry diets. Ideally, such alternatives should be tolerant to drought conditions and poor quality soils.

Over the past 25 years, a crop-breeding group at the University of New England (UNE) has been developing cultivars of triticale, a hybrid cereal grain produced by crossing wheat (*Triticum aestivum*) with rye (*Secale cereal*) using recently developed plant breeding techniques by a crop breeding group at UNE. The efforts of this group have produced cultivars of triticale which are not only high in digestible energy content but also high-yielding and tolerant of acid soils (UNE, 2008). Previously, Scanlan (2005) reported that, for a wheat-related grain harvest, one of the group's experimental triticale crops had broken through the 10-tonne-per-hectare barrier. In comparison, Australia's average wheat yield was only two tonnes per hectare. In Europe, wheat yields do not exceed 6 to 8 tonnes per hectare. The poultry industry would benefit from the use of such triticale cultivars as an alternative source of energy. However, studies are required to determine the nutrient composition of triticale cultivars and the efficiency of growth of poultry on diets based on them. This will hasten their adoption by the poultry industry and lead to improvement in the quality of the triticale cultivars. To date only a single experiment has been completed on four of the cultivars developed at UNE and the results suggest that there was a variability between the cultivars in terms of nutrient values, relative to wheat (Elangovan *et al.*, 2009).

1.2 THE RESEARCH OBJECTIVES

The main objective of this study was to evaluate the relative nutritive value of different new cultivars of high-yielding triticale and explore their potential as a digestible energy and protein

source for poultry, leading to the production of low-cost diets. This general objective has the following specific aims:

- To evaluate the nutrient composition of the new cultivars of high-yielding triticale;
- To determine the metabolisable and net energy contents of diets based on the new cultivars of high-yielding triticale;
- To assess the replacement value of the triticale cultivars in conventional wheat-based diets;
- To examine the possibility of improving the nutritive value of the triticale grain by use of microbial enzyme preparations, and
- To examine the physiological and production responses of broiler chickens to diets containing triticale.

CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

Cereal grains are the most important energy sources in poultry feeds. One-third of the global grain production is used for animal feeding, of which about two thirds is used by the poultry industries (Speedy, 2003). Some cereal grains such as maize, sorghum, millet, triticale, and oats are grown predominantly for animal consumption, whereas others, including wheat, barley and rice are grown mainly for human consumption but grains not meeting market specifications are frequently fed to livestock. Maize and wheat are widely used as ingredients in poultry diets. The high cost of production and hence increasing prices of maize and wheat, as well as sorghum and barley in Australia, have necessitated the search for other ingredients to replace these cereals, partly or wholly, in poultry diets. The high-yielding potential, stress tolerance (especially drought tolerance) and disease resistance of triticale (Todorov, 1988), makes it a valuable alternative in poultry diets.

Triticale is the first man-made cereal grain crop species resulting from the hybridisation of wheat (genus *Triticum*) with rye (*Secale*). In recent years, much breeding work has been conducted to produce new cultivars that carry over the valuable agronomic characteristics of their parents, such as the productivity and disease resistance of wheat with the vigour and hardiness of rye. However, although triticale has a chemical composition that is relatively similar to wheat, the higher content of anti-nutritional factors, such as soluble non-starch polysaccharides (NSP), arabinoxylans in particular, and phytate, means that, compared to wheat or maize, it has potential limitations for use in poultry diets. Supplementation of triticale-based diets with microbial enzymes (e.g. xylanase and phytase) may be a practical solution to improve the nutritive value of triticale.

2.2 FEED INGREDIENTS FOR POULTRY

There are four main components that can be combined to provide a balanced poultry diet. The first is an energy source and, although energy is not regarded as a nutrient, it is the most costly part of the diet. In the poultry meat industry, the feed constitutes up to 70% of the total cost of production (Cooke, 1987), and in general, the requirement for energy constitutes about 60 to 70% of that cost (Skinner *et al.*, 1992; Kleyn, 2007). It is, therefore, the cost and availability of feed ingredients that are the crucial factors. The other three components are protein, vitamins

and minerals, which are categorised as nutrients. In short, Leeson and Summers (2001) stated that a balanced diet is prepared based on cereals for energy, vegetable and animal protein sources for amino acids and additives containing vitamins and minerals in order to meet the bird's nutrient needs.

2.2.1 Energy sources

Energy sources constitute the largest component of poultry diets, and carbohydrates represent the major energy component in poultry diets. Klein *et al.* (2001) reported that 70% of the bioavailable energy comes from carbohydrates, of which starch is the most important fraction that is useful to the animal. The main sources of carbohydrates in poultry diets are supplied by cereal grains. The cereal grain tissue is predominantly starchy endosperm, where starch is stored in an insoluble form (Evers *et al.*, 1999). Besides being high in carbohydrates, energy feedstuffs have two other characteristics, i.e. they have less than 20% crude protein (CP) on a moisture-free basis and a low crude fibre concentration of less than 18% (Myer, 2005).

The cereal crops cultivated around the world that have the highest total world production in 2010 (FAOSTAT Database, 2012) are maize, rice, wheat, barley, sorghum, oats, triticale and rye. These grains are nutritionally rich and provide carbohydrates, protein and various micronutrients, particularly some B vitamins, vitamin E and minerals. Cereal grains are mainly used in animal diets as energy sources and specifically for commercial poultry diets.

Ravindran and Blair (1991) pointed out that some roots and tubers, fruits and their by-products can be used as cereal substitutes. The decision on which grain or other energy source to use for supplying the energy requirement of a diet is important. Among the cereals mentioned, maize is the major contributor of metabolisable energy (ME) in most poultry diets (Leeson and Summers, 2009). However, significant amounts of sorghum, wheat, barley and rice or rice by-products are also used in poultry diets when their price and availability allow for their inclusion in the diet. The use of other cereals, including oats, rye, triticale and millet, is relatively minor.

Fats and oils are also used as energy sources. Fats are relatively inexpensive and are a dense form of energy, containing about 38 MJ/kg DM, whereas carbohydrate and proteins contain about 17 MJ/kg DM. The most important lipids come from animals and vegetable or a blend of both.

2.2.2 Protein sources

Protein is another critical nutrient, particularly for young, rapidly growing animals and high-producing adults, although under some conditions it becomes secondary to energy (Pond *et al.*, 2005). Protein supplements are typically defined as those feedstuffs containing 20% or more CP (Chiba, 2005). A wide variety of protein sources are used as ingredients for poultry diets. Soybean meal is the most widely used vegetable protein supplement in typical poultry feed formulations, although in some parts of the world, this is replaced by canola meal, pea or sunflower meal. The animal protein sources that contribute to the protein requirement of the bird include fish meal, meat meal, and meat and bone meal (Ravindran and Blair, 1992, 1993).

There are at least three reasons for optimising the utilisation of protein concentrates for poultry. Firstly, essential amino acids are vital nutrients for both rapidly growing meat-type birds and high-producing laying hens; secondly, protein sources are usually more expensive than energy feedstuffs; and thirdly an appropriate balance of dietary amino acids lessens the production of nitrogenous waste products by the birds, and as a consequence, the nitrogen released into the environment is reduced (Elkin, 2002).

2.2.3 Micro ingredients

Mineral supplements

Although minerals are needed only in small amounts in a poultry diet, they are vital to the bird. There are now twenty-nine elements that have been identified as being required by most animal species. Based on traditional classification, seven elements are regarded as macro, required at more than 100 ppm, and 22 are trace or micro minerals, which are required at rates below 100 ppm; some are even required in ppb values (McDowell, 2003). Leeson and Summers (2001) noted that the minerals which are of particular nutritional importance in the body of the chick are Na, K, Cl, Ca, P and Mg. Others that are also essential are Mn, Fe, Cu, Zn, I and Se.

The minerals do not yield any energy but they have various important roles in the bird's metabolism and development. The body generally requires the minerals for the formation of the skeleton, as components of assorted compounds in the body, as cofactors of enzymes, and for the maintenance of osmotic balance within the body. Ca and P are two of the most abundant minerals in the bird's body, due mainly to their major involvement in bone formation (Nelson *et al.*, 1965). In addition, Ca takes part in blood clotting (Hays and Swenson, 1985) and P is

involved in energy metabolism and as a structural component of many important metabolites (Leeson and Summers, 2001). Sodium, K, Mg and Cl function with phosphates and bicarbonate to maintain the homeostasis of osmotic pressure and pH throughout the body (Hays and Swenson, 1985; Murray *et al.*, 2000).

Trace minerals also play important roles in numerous biochemical functions in avian and mammalian species, for example being essential components of many enzymes (Vallee and Auld, 1990), and having both structural and catalytic functions in metalloenzymes (O'Dell, 1992). Copper, for instance, is a trace mineral, which is an indispensable component of uricase, ascorbic acid oxide and ceruloplasmin. Another example is zinc, which is essential for carbonic anhydrase and carboxypeptidase (Cousins, 2006). Moreover, trace minerals are required for normal immune function (Goswami *et al.*, 2005), as well as being essential for growth and skeletal development (Brandão-Neto *et al.*, 1995). In poultry, Zn deficiency causes a reduction in weight gain, skeletal malformations, poor bone mineralisation, and immunological dysfunction (Blamberg *et al.*, 1960; Kidd *et al.*, 1996).

Owing to the shortage of certain minerals in concentrates or feedstuffs, some diet supplementation is required to meet needs. It is a common practice to include 0.25 to 0.5% common salt (NaCl) in most commercial poultry feed formulations; however, the actual requirement depends on the species (NRC, 1994). Many feedstuffs are deficient in Ca and/or P as large amounts of these minerals are needed by the birds. Some widely used supplements in poultry diet are bone meal, mono-, di- or tri-calcium phosphate, oyster shell and limestone (Leeson and Summers, 2001; Pond *et al.*, 2005). In order to meet the requirement for trace minerals, a mineral premix is regularly included in commercial diet formulation. Tian *et al.* (2001) suggested that the requirements may vary widely owing to environmental conditions, such as temperature, humidity, management and severity of stress as well as the physiological status of the animal. Therefore, it is not easy to determine the optimum requirements of trace minerals in commercial diets; however, numerous efforts have been made to determine such levels (NRC, 1994).

Vitamin supplements

Vitamins are essential for the normal growth and development of multicellular organisms. The general functions of these nutrients for animals are to facilitate chemical reactions that are involved in the development of skin, bone and muscle (Leeson and Summers, 2001). As with

minerals, there are two sources of vitamins for poultry. Firstly, vitamins are natural components of the ingredients used in the diet, and, secondly, they can be added in a concentrated form as a supplement.

Vitamins are generally classified into two groups: vitamins A, D, E and K are grouped as fat-soluble vitamins, while the second group consisting of B-complex vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, cobalamin, folic acid) and vitamin C (ascorbic acid) are water-soluble (Pond *et al.*, 2005). Vitamin C is one of the vitamins that can be synthesised by poultry; it is, accordingly, not considered to be an essential dietary nutrient. Nevertheless, Pardue *et al.* (1985) found that the inclusion of ascorbic acid in the diet can improve growth, feed efficiency and liveability of broilers in acutely hot conditions.

Most of the ingredients included in the diet contain some vitamins; however, the concentration of vitamins varies greatly owing to factors such as harvesting time, processing, storage conditions and the species or parts (seed, leaf or stalk) of the plant (Pond *et al.*, 2005). Nowadays, to meet the vitamin requirements of poultry, premixes, composed of vitamins and micro minerals, are routinely supplemented (Leeson and Summers, 2001).

Non-nutritive feed additive

In modern commercial feed formulations, the use of a diverse range of non-nutritive additives has been widely applied. The additives may not be physiologically essential, but their usage has been demonstrated to lead to improved performance and health (Ravindran, 2011). Common feed additives used in poultry diets include feed binders, antimicrobials, antioxidants, antifungals, pigments, emulsifiers, pH control agents, probiotics and enzymes (Wenk, 2000).

2.3 CEREAL GRAINS

Cereal grain species are defined as "flowering plants of the grass family (*Poaceae* or *Gramineae*), whose seeds are used for food" (Wrigley, 2004). They are grown extensively on all continents and have been well adapted to tropical and temperate climate zones. Australian tropical cereals include maize, sorghum and rice (Lovett and Lazenby, 1979), and temperate cereals include wheat, barley, oats and rye (Lazenby, 1987), as well as triticale (Brown, 1989). As an energy source, cereal grains also make up the bulk of any diet formulated for birds. In Australia, the common cereals used for broiler diets are wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*) and barley (*Hordeum vulgare*), with some diets including a minor utilisation

of maize (*Zea mays*), rye (*Secale cereale*), oats (*Avena sativa*) or triticale (*X Triticosecale Wittmack*). The world and Australian cereal grain production is shown in Figure 2.1.

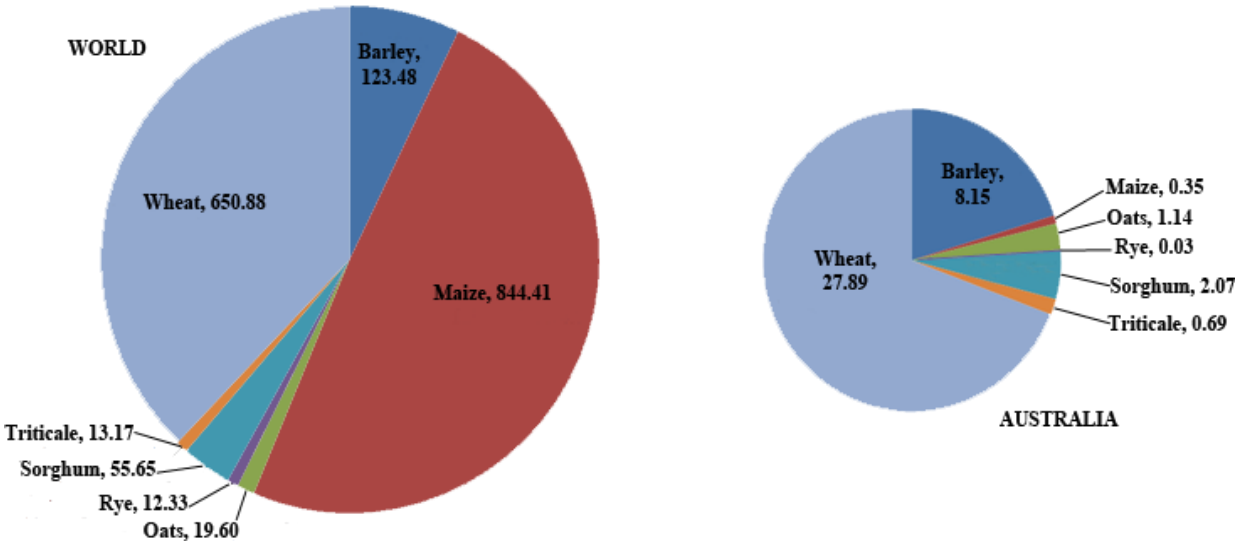

Figure 2.1 2010 world and Australian production of the common cereal grains used for poultry feeding (million tonnes) (ABARES, 2011; FAOSTAT Database, 2012).

2.3.1 Tropical cereals

Despite their tropical origin, sorghum and maize, the summer-growing crops, can be grown in areas with a relatively short summer season, provided that an adapted cultivar is used. Maize, for instance, is divided into three types based on the growing region, tropical maize (growing in warmer environments, located between 30ºN and 30ºS latitudes), an intermediate type (subtropical maize) that is grown between 30º and 34º latitudes (subtropical), and temperate maize which is grown in cooler climates beyond 34ºN and 34ºS (Xu and Crouch, 2008). However, in the following review, maize and sorghum will be regarded as tropical cereals, as more than 80% of Australian maize and sorghum for grain production is grown in those regions (Australian Bureau of Statistics, 2008).

Maize (Zea mays)

Maize, or corn, as it is called in some parts of the world including the US, is the number one cereal in the world in terms of production. Maize is widely grown throughout the world and is produced in greatest volume of all the cereals, with more than 844 million tonnes being

produced globally in 2010, 29.70% more than the second-ranked cereal, wheat (650.88 million tonnes). Furthermore, the production of maize has increased by approximately 37% since 2001, whereas the production of wheat has remained relatively constant. Barley and sorghum production has also decreased relative to maize (FAOSTAT Database, 2012). In contrast to world production, maize production in Australia is only 0.35 million tonnes, or less than 0.05% of the world maize production in 2010 (ABARES, 2011), with about 10% of that being exported.

Globally, maize is the principal component in the diets of intensively reared poultry. It has been utilised as a major energy component in poultry diets because of its high available energy content and low soluble NSP, which are anti-nutritive factors (Iji *et al.*, 2003), and in addition, the ME of maize in broiler diet is about 14 MJ/kg (NRC, 1994). The starch content of maize varies from 64 to 80% and this leads to a higher apparent metabolisable energy (AME) than wheat and barley (Eliasson and Larsson, 1993). Nevertheless, there are differences of more than 2 MJ/kg in AME from batch to batch, and consequently, the generic energy matrix values often listed in feeding tables for maize can be inaccurate (Cowieson, 2005). The NSP content of maize is about 9.5%, less than that of wheat and barley (Eliasson and Larsson, 1993). Furthermore, Choct (2006) reported that the amount of arabinoxylan, a soluble NSP found in maize, is negligible compared to the higher levels in wheat. Connor *et al.* (1976), who investigated the nitrogen-corrected AME (AMEn) values for 20 Australian maize varieties, found that the values ranged from 15.5 to 17.0 MJ/kg DM (mean value=16.3 MJ/kg). Cowieson (2005) explained that the main reasons for this difference in energy values were the higher starch content of maize (more than 600 g/kg) and the relatively lower concentration of soluble NSP than wheat. This author added that maize contains a relatively low concentration (about 80 g/kg) of CP compared to wheat and barley. Furthermore, the total P and phytate-P concentrations in maize are about 2.8 g/kg and 1.9 g/kg, respectively (Eeckhout and De Paepe, 1994).

Sorghum **(Sorghum bicolor)**

The global production of sorghum is far less than that of maize, wheat and barley; however, sorghum is ranked third in grain production in Australia. It is used for human food and livestock feeding (Gualtieri and Rapaccini, 1999). There is a wide variety of sorghum cultivars grown in various regions throughout the world. Sorghum is more drought-resistant than maize or wheat. This factor makes it a viable crop in the tropical and sub-tropical areas that have low rainfall

(Hulse *et al.*, 1980; Doggett, 1995; Gualtieri and Rapaccini, 1999). In most cultivars of sorghum, the CP concentration varies between 8.8 and 15%, slightly higher than maize, with a very uniform profile of essential amino acids (Nelson *et al.*, 1965; Douglas *et al.*, 1990) and the ME value is about 5% less than that of maize, which is about 13.4 kcal/kg (NRC, 1994). A small variation of AME and AMEn for Australian low-tannin sorghum has been reported recently by Robertson and Perez-Maldonado (2006) with concentrations ranging from 15.2-16.2 and 14.7-15.5 MJ/kg DM, respectively. These variations can be compared to the ME concentrations reported by Walker (1999), and Gualtieri and Rapaccini (1999), which ranged from 11.0 to 16.0 MJ/kg DM.

Sorghum contains other anti-nutritive factors that can negatively influence its nutritive value. Sorghum grain (also called 'milo') contains some phytate and condensed tannins. The protein, called kafirin, is low in lysine and less digestible, while phytate and tannin have the capacity to complex protein in the gut and reduce protein digestibility and intestinal absorption of dietary and endogenous amino acids (Selle *et al.*, 2010). However, Australian sorghum varieties are typically low in tannins (Black *et al.*, 2005) and have relatively low soluble NSP compared to maize and rice (Choct and Annison, 1990). Nyachoti *et al.* (1997) concluded that its low tannin content allows sorghum to become an alternative cereal to maize for inclusion in diets for poultry. In addition, its nutritive value is close (essentially 95-96%) to that of maize (Leeson and Summers, 2001).

2.3.2 Temperate cereals

Australian temperate cereals include wheat, triticale, barley and oats which are grown during the winter and spring (Lopez-Castaneda and Richards, 1994). Wheat and barley are considered as primary crops in Australia because they have been widely used for a long time, while the other two, oats and rye, which are of more recent introduction, are described as secondary crops that originally grew as weeds, but were later cultivated (Lazenby, 1987). Triticale varieties were introduced into Australia in the early 1970s as experimental lines for evaluation (Cooper *et al.*, 2004).

Wheat **(Triticum aestivum)**

Wheat is widely grown around the world under various climatic conditions and has become a major grain in Australia. Globally, in 2010, wheat was the second most produced cereal after maize with a world production of over 650 million tonnes (FAOSTAT Database, 2012), while

in Australia, it is the most cultivated grain crop, representing over 69% of the cereal grain commonly used in poultry diets (ABARES, 2011), which the national production was just under 28 millions tonnes. In December 2011, about 66% of national total production was exported, and the rest production was for domestic use (ABARES, 2011). The largest consumer of domestic wheat is for the livestock industry, which is consuming an average 53% over the last 5 years, while 21% of that figure was used in poultry industry (DPIW, 2012).

In Europe, wheat contributes up to 65% in finisher diets for broiler chickens (Gutierrez-Alamo *et al.*, 2008). In Australia, wheat can on occasions account for more than 70% of the broiler chicken diet (Annison, 1993). Furthermore, wheat grain is often used for poultry feed under certain conditions such as surplus production, being unsuitable for the market owing to small grain size, low demand or low price.

***Barley* (Hordeum vulgare)**

Barley grain ranks third among the cereals for feed grain production, with a world output of over 123 million tonnes in 2010 (FAOSTAT Database, 2012). In Australia, barley is the second largest grain crop, comprising about 20.2% of the total grain produced in Australia (ABARES, 2011), and it is used principally for animal feeding, but also for human consumption and to make malt used in beer brewing. Barley is the favoured grain for cultivation in many parts of the world owing to its resistance to drought and ability to mature in climates with a short growing season. However, its use in poultry diets is limited by the considerable amount of fibre contained in the grain (Svihus and Gullord, 2002).

***Rye* (Secale cereale)**

Rye is a minor crop compared to maize or wheat in terms of world production. The production of rye has decreased by approximately 50% since 2001 (FAOSTAT Database, 2012). However, 33% of the global rye production is used for food and about 15% for industrial use, including whisky production. In Australia, the production of rye is negligible, and it is used mostly for livestock feeding with a small amount for human consumption. Its use in poultry diet is very limited owing to an abundance of anti-nutritional factors. It is; however, the most dependable cereal grain for winter grazing, especially in the USA. Rye has greater cold tolerance, quicker growth at low temperature and more seasonal forage production than wheat, oats, barley or triticale (Bruckner *et al.*, 1999).

***Oats* (Avena sativa)**

Oats ranks fifth in world cereal production for feed grain, following maize, wheat, barley and sorghum. According to McGee (2004), 95% of the crop is fed to animals. Oats is used for forage and fodder, straw and bedding, hay, haylage, silage and chaff in many parts of the world (Welch, 1995); however, it is not widely used for feeding pigs or poultry owing to its high fibre content (Pond *et al.*, 2005). In addition, Farrell *et al.* (1991) reported that the levels of β-glucans in hulled oats are relatively high. On the other hand, Ougham *et al.* (1996) suggested that naked or hull-less oats (cultivar *Avena nuda*) can be used as alternative ingredient in poultry diet. This cultivar is similar to the species *Avena sativa* (common oats), but has a dominant gene which gives rise to a phenotype with a non-lignified husk.

***Triticale* (X Triticosecale *Wittmack*)**

Triticale was the first human-developed cereal, from crosses between wheat as the female parent and rye as the male parent and its development dates back to 1875 (Müntzing, 1979). Since then, there have been many efforts by breeders to develop a high-yielding new species that combines the positive traits of both parents, for instance the height and bread quality requirement from wheat, and the winter-hardiness and disease resistance from rye (Merker, 1971; Gregory, 1973; Larter, 1973; Sanchez-Monge, 1973; Mackowiak and Lapinski, 1985). Modern triticale grain resembles wheat more than rye morphologically; however, its weight is slightly lower than that of wheat (NRC, 1989; Varughese *et al.*, 1996; Boros, 2002; van Barneveld, 2002).

In terms of world grain production, less triticale is grown than oats but slightly more than rye, however, in Australia, more triticale is grown than maize (ABARES, 2011; FAOSTAT Database, 2012). Triticale is predominantly used as feed grain for livestock industries (Bird *et al.*, 1999; Salmon *et al.*, 2004) and only a small portion is used for human consumption (Mergoum *et al.*, 2004; Oettler, 2005). All Australian triticale is produced for domestic use, unlike wheat, barley, oats and sorghum, some of which are exported.

2.4 NUTRITIVE VALUE OF TEMPERATE CEREALS

The nutritional values of feed ingredients previously described, will be reviewed in this section, however the nutritive value of triticale will be detailed reviewed in Section 2.5.2.

2.4.1 Carbohydrates

Carbohydrates are the largest component of the solid contents of cereal grain. The remaining components being mainly proteins, lipids, minerals, vitamins and often anti-nutritive factors. Carbohydrates are composed of three large components, namely starch, NSP and free sugars including mono-, di- and oligosaccharides. However, carbohydrates make up 80 to 90% of cereal grains, but the proportions of these components do vary depending upon the grain type, cultivar and geographical location (Annison, 1989; Scott *et al.*, 1998; Choct *et al.*, 1999a; Steenfeldt, 2001). In cereal grains, as well as in other plant tissues, carbohydrates are localised in three places, firstly in the cell wall with especially thickened walls in supporting tissues of husk and seed coat; secondly in plastids, where starch constitutes the largest proportion of carbohydrates in all cereals, and thirdly, in vacuoles or the cytoplasm (Alais and Linden, 1991).

Dietary fibre is present as components of the plant cell wall (Alais and Linden, 1991). These include cellulose, hemicellulose, pectin, 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. The biological availability of protein, minerals and other nutrients, such as vitamin B_1 in rice, may be reduced by its 'fibre constituents' (Torre *et al.*, 1991). The term *fibre* is used to describe the NSP and associated fibrous structures in the feed. Starch is composed of two carbohydrate components, both high molecular weight polymers of glucose. These are amylose and amylopectin. In most starches, 20-25% of the starch is amylose, while more than 70% is amylopectin. The ratio of amylose to amylopectin influences the degree to which starch is digested by poultry. Amylose is an almost linear polymer glucose (~99% of α(1-4) and only ~1% of α(1-6) glycosidic linkages), and amylopectin consists of a heavily branched polymer (with ~95% of α(1-4) linked glucose chains with ~5% of α(1-6) linked branching glucose chains, forming an unstructured gel without crystalline structures. High amylose starches are poorly digested compared with starches containing mainly amylopectin (Black, 1999). However, because of its tightly packed molecular structure, amylose is more resistant to digestion than amylopectin and is therefore an important form of resistant starch (Sinovec and Markovic, 2005).

Starch in wheat can comprise up to 73% of its DM content (Carré *et al.*, 2002; McCracken *et al.*, 2002). Most of the AMEn of wheat depends on the utilisation of the starch fraction (content and digestibility) as it is the largest contributor to the energy supply from the grain, but the important consideration is the correlation between starch digestibility and AME, rather than

between starch content and AME (Gutierrez-Alamo *et al.*, 2008). The amylose and amylopectin content of wheat varies between 21.1 and 33.5%, and 33.5 and 45.4% DM, respectively (Annison, 1990). In general, NSP in cereal grains are predominantly arabinoxylans (pentosans), β-glucans and cellulose. Choct (2006) reported that arabinoxylans are the major soluble NSP in wheat, being 1.8% DM, while β-glucans are 0.4% DM.

Starch is also the major component of barley, varying between 51.3 and 64.2% (Holtekjølen *et al.*, 2006). β-glucans and arabinoxylans are the major fibre components in barley grain and their concentration in barley is two to five times higher than in wheat (Choct, 2006; Holtekjølen *et al.*, 2006). Leeson and Summers (2009) reported that most varieties of barley contain 4-7% β-glucans, although in dry growing conditions the content can increase to 12-21%. The same result was reported by Choct (2006) who found that the total soluble NSP was dominated by β-glucan (3.6% DM). Therefore, in order to improve the nutritive value of barley for poultry, supplementation with β-glucanase has, for more than 20 years, become a practical solution to this problem (Hesselman and Åman, 1986; Campbell *et al.*, 1989). Among the cereal grains, rye has the highest content of soluble NSP, which are dominated by arabinoxylans (3.4% DM) and β-glucans (0.9% DM) (Choct, 2006).

The 55.4% starch content of oats is lower than in the other temperate cereals (Józefiak *et al.*, 2006). The soluble NSP content of oats is dominated by β-glucans (2.8% DM), followed by arabinoxylans (0.8% DM) (Choct, 2006).

The starch content of triticale varies between 54.5 and 60.7%, with the concentration of amylose ranging from 26.3 to 29.6%. Total insoluble NSP content in triticale is higher than in wheat but the soluble NSP are less than in wheat. Soluble arabinoxylan and β-glucan contents of triticale are 0.7 and 0.58%, whereas in wheat, contents are 0.75 and 0.70%, respectively (van Barneveld, 2001). Flores *et al.* (1994b) in a study of 18 varieties of triticale in Spain, reported that starch content of triticale ranged from 56.3 to 62.9% DM, and the water-soluble pentosan contents ranged from 2.77 to 5.09% DM.

2.4.2 Energy content

Most countries use ME content of feed ingredients as the evaluation parameter for formulating diets for poultry. It can be expressed as AME or AMEn. However, there is wide variation in AME within and between grain species used for broiler chicken diets. Black (2001) reported

that AME variation is related to the gross chemical composition of the grain. The other factors include endosperm cell wall characteristics, grain hardness, fatty acid content and composition, relative portions of amylose and amylopectin in starch, chemical and physical nature of the protein-starch matrix and phenolic bonds with lignin, polysaccharides and protein.

The AME of Australian wheat ranges from 10.0 to 15.9 MJ/kg DM (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Choct, 1995; Hughes *et al.*, 1996). The total soluble NSP of wheat are dominated by arabinoxylan, β-glucan and galactan, and concentrations of these are higher than in triticale, sorghum and maize (Choct, 2006). Barley grain has a high fibre content and its energy yield as AME for broiler chickens is a low 11.9 MJ/kg (DM) (Choct and Annison, 1990). In addition, Kocher *et al.* (1997) reported that AME of Australian barley also varies widely between 10.4 and 12.4 MJ/kg DM, and increases with inclusion of commercial β-glucanase (13.2-15.5 MJ/kg DM). Choct (2006) observed that rye, like barley, has low AME (about 11.0 MJ/kg) owing to the high content of soluble NSP. The AME value of oats is lower than that of wheat and maize. Metayer *et al.* (1993), investigating French oats varieties, found that the AME of black oats varied between 10.5 and 11.4 MJ/kg, while white oats ranged from 11.6 to 12.4 MJ/kg. Hughes and Choct (1999) reported that the AME values of three Australian oats ranged from 11.8 to 12.4 MJ/kg DM, furthermore the AME values of Australian wheat, barley and sorghum ranged from 10.4 to 15.9, 10.4 to 12.2 and 14.9 to 15.8 MJ/kg DM, respectively.

2.4.3 Protein and amino acids

There is great variability in the CP content of wheat throughout the world. The CP of nine cultivars in Czechoslovakia and nine local wheat cultivars in Poland were investigated by Heger *et al.* (1990) and Barteczko *et al.* (2009), respectively. In the Czechoslovakian cultivars, CP content ranged from 12.7 to 13.8% (DM), while in the Polish cultivars the CP value ranged from 11.9 to 15.1% (DM). Mollah *et al.* (1983) in a study on 22 Australian wheat cultivars reported a CP content of between 11.4 and 18.0% (DM). Parsaie *et al.* (2006) evaluated the CP value of 15 local Iranian wheat cultivars and found a range of 10.4 to 15.4% (DM). A study by Boila *et al.* (1996) in Manitoba on three Canadian varieties from 12 locations for three consecutive years found that the mean CP was 16.0% (CV 10.6%). As with wheat, the CP content in barley varies. Ravindran *et al.* (2005) reported that the CP value of barley (as received) is about 9.7%, while Leeson and Summers (2009) found that it is usually about 11 to 12%, but it can be as high as 14 to 16%. In barley, lysine is the first-limiting amino acid, followed by threonine, methionine and histidine (McDonald *et al.*, 2002). The protein content

of rye is around 12.1% (NRC, 1994) and the amino acid composition is relatively similar to wheat (McDonald *et al.*, 2002), but higher in lysine and slightly less in sulphur amino acids and trypthophan. Although the nutrient composition of rye is essentially similar to that of wheat and maize, its nutritive value for poultry is poor owing to the presence of various anti-nutritional factors (Leeson and Summers, 2009). Triticale has a higher yield per hectare than rye or wheat, making it of agronomic interest in areas of the world not suitable for maize production (Leeson and Summers, 2009). Furthermore, rye has a similar nutrient composition to wheat, and is higher in some amino acids and in protein content.

2.4.4 Lipids

Lipids yield 2.25 times the energy of carbohydrates and protein per unit weight, provide essential fatty acids, add flavour, improve the efficiency of feed conversion and reduce the dustiness of milled grains. The crude fat content of wheat also varies between cultivars. The CF content of Polish wheat cultivars ranged from 1.06 to 2.58% and 1.38 to 2.22% (DM) as reported by Barteczko *et al.* (2009) and Lasek *et al.* (2011), respectively. This can be compared with the Czechoslovakian cultivars with a variation of from 1.65 to 2.03% (DM) (Heger *et al.*, 1990). The following is the total lipid content of the various cereal grains: oats, 5 to 9%; barley, 3.3 to 6.4%; rye, 2.0 to 3.5%; wheat, 2.3 to 2.5%, and triticale 2.0 to 2.3%, (Morrison, 1978; Youngs, 1986; Chung and Ohm, 2000).

In brief, the chemical composition and nutritional value of wheat are most likely to be similar to other cereal grains, and are variable, depending upon the grain variety. Factors causing this variability include the growing conditions, post-harvest storage, chemical composition and animal factors (Gutierrez-Alamo *et al.*, 2008).

2.4.5 Nutrient digestibility

Starch is the major energy-yielding component in poultry diets and is presumed to be almost completely digestible in poultry at all ages (Wiseman, 2006). Nevertheless, incomplete digestion of the starch in wheat has been observed in broiler chickens with values for 20 Australian wheat varieties observed by Annison (1990) varying from 93.5 to 98.2%. Rogel *et al.* (1987) found that digestibility of starch was poor when the birds were fed un-pelleted wheat diets. Hesselman and Åman (1986) reported that wheat starch digestibility was improved by the inclusion of β-glucanase in the diet. According to Moran (1982a), starch digestibility in wheat is a function of granular surface area, starch structure and degree of crystallinity. Granule

size differs among feedstuffs, with small granules usually digested more rapidly than larger granules (Franco *et al.*, 1992).

The presence of soluble NSP and phytate can also reduce the digestibility of starch. The soluble NSP in the diet increase digesta viscosity, which can reduce starch digestibility (Choct and Annison, 1990; Classen, 1996; Refstie *et al.*, 1999) while the presence of phytate affects starch digestion through the formation of tertiary complexes with starch or binary complexes with calcium, a cofactor required for α-amylase activity (Cowieson and Adeola, 2005). Moreover, increased activity of microflora in the gastrointestinal tract of the chicken will depress energy utilisation (Choct *et al.*, 1996), apparent protein digestibility (Smits *et al.*, 1997) and availability of amino acids (Steenfeldt *et al.*, 1995) in the diet.

Dietary supplementation with enzymes has been widely used to increase nutrient utilisation. Liu *et al.* (2011) reported that inclusion of xylanase in the diet can help to degrade NSP and consequently improve utilisation of diet components, so that the digestibility of hemicellulose and energy increased by 20% and 62%, respectively. Singh (2008) reported that phytic acid reduced the bioavailability of P, Ca, Mg, Zn and many other trace elements. The liberation of these minerals may occur by hydrolytic splitting of the ester bond of phytic acid through the effects of various types of feed processing and/or by the action of phytase from animal, plant or microbial sources. The presence of endogenous phytase in plant feedstuffs is relatively low and so phytase supplementation may improve P retention in practical diets for broiler chickens (Barrier-Guillot *et al.*, 1996) and in addition, may enhance protein availability (Farrell *et al.*, 1993), increase digestibility of all amino acids except methionine (Kornegay, 1996), increase AME (Ravindran *et al.*, 2000), and improve availability of calcium for broiler chickens (Schöner *et al.*, 1993; Yi *et al.*, 1994; Sebastian *et al.*, 1996; Singh and Khatta, 2003) as well as copper (Aoyagi and Baker, 1995) and magnesium (Mohanna and Nys, 1999).

2.5 TRITICALE

2.5.1 Volume of production

Poland is the largest producer of triticale grain, with over 31% of the world's output, followed by Germany, France, Belarus and Australia, which produced respectively, 16.7, 15.6, 9.5 and 3.8%, of the global triticale grain production in 2010 ((FAOSTAT Database, 2012). There are 23 other countries that produced from 0.07 to 1.35% of world production (Figure 2.2). However, in terms of yield, Belgium and Chile are the countries with the highest triticale yield,

i.e. 6.57 and 6.15 tonnes/ha, respectively in 2010, compared with triticale yield in Australia, which was 1.60 tonne/ha.

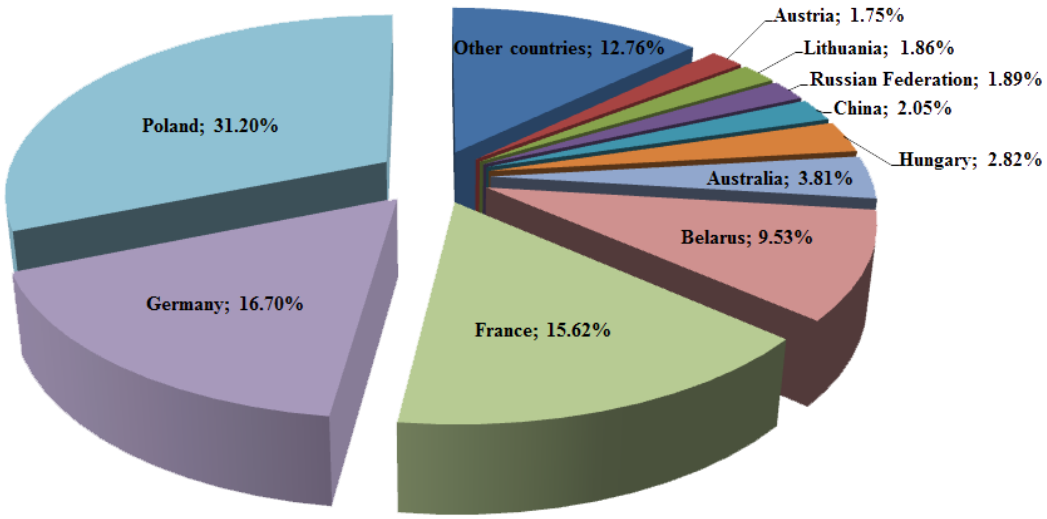

Figure 2.2 World triticale grain production (%) in 2010 (FAOSTAT Database, 2012).

However, as the fifth ranked country in world triticale grain production, Australia contributes over 3.8% of the world's triticale grain production, mainly for domestic use. Very small amounts of triticale are used for human consumption (Cooper *et al.*, 2004). The authors added that about 30% of the triticale grain in Australia is kept on farm for use locally, and the remainder is sold to dairy farms or piggeries, feed-mills that process raw materials to produce compound feeds, or to grain-handling organisations that provide bulk storage and market larger parcels of grains. Nevertheless, there is apparently no official report so far for the quantity of triticale grain used for poultry feeding in Australia.

In Australia, the temperate regions located in the southern part of the continent are more favourable for triticale production (Fig. 2.3).

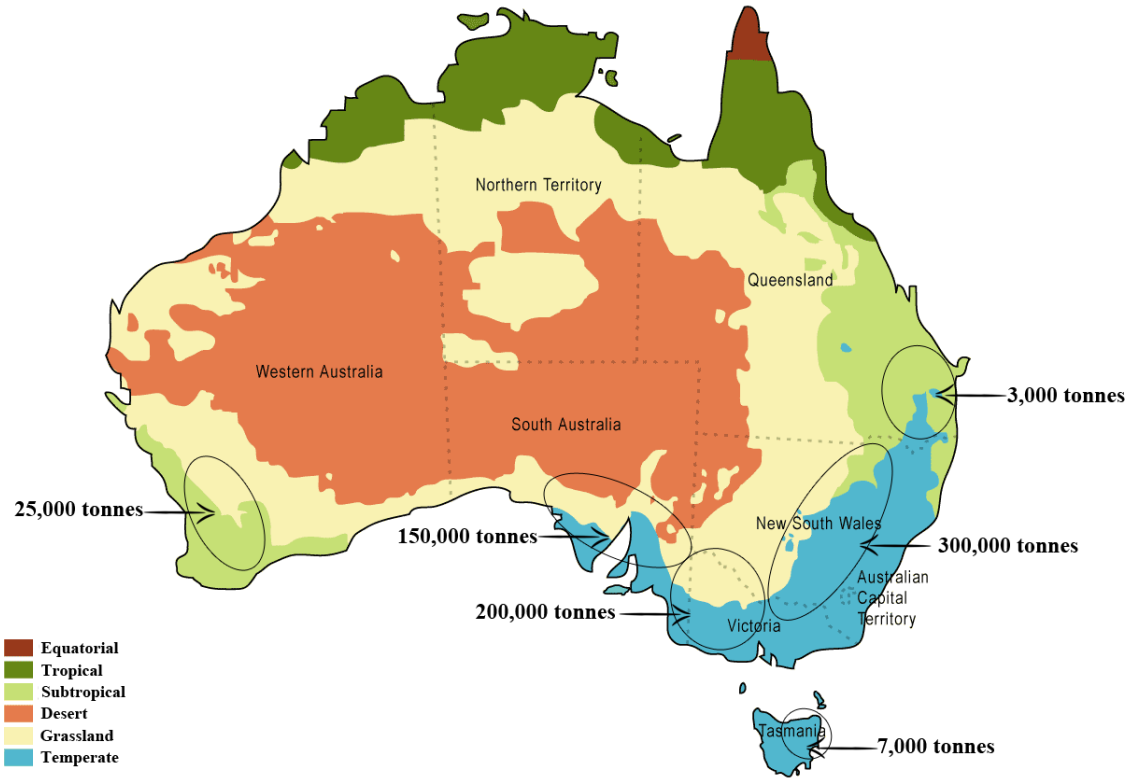

Figure 2.3 Production of triticale grain by State, 2010-2011 (ABARES, 2011).

Picture adapted from Stern *et al*., (2000) and van Barneveld (2002)

According to ABARES (2011), Australia produced about 685 tonnes of triticale grain in 2010 to 2011 (Figure 2.3). Triticale is grown across Australia with the most produced in New South Wales (300,000 tonnes), Victoria (200,000 tonnes) and South Australia (150,000 tonnes). Smaller quantities are produced in Western Australia (25,000 tonnes), Tasmania (7,000 tonnes) and Queensland (3,000 tonnes). The growing regions correspond with the bulk of Australia's intensive livestock production, making triticale available to most feed mills. Within these regions, some triticale is grown for grazing, and hay and silage production.

2.5.2 Nutritive value of triticale

Since triticale was introduced to Australia and utilised as an ingredient in diets for poultry over 30 years ago (McKenzie and Farrell, 1980), some researchers have reported that the nutritive value of Australian triticale varieties has increased as a result of breeding and development programmes to improve its agronomic characteristic (Farrell *et al.*, 1983; Johnson and Eason, 1988; Jones *et al.*, 2000).

Energy content

A wide range of AME values for triticale (8.6-14.1 MJ/kg in chicks) was reported by Shingari *et al.* (1976) who studied the Indian triticale cultivars. In France, Metayer *et al.* (1993) reported that the AME content of triticale in poultry was, on average 14.27 ± 0.19 MJ/kg DM, in tests of 130 triticale varieties samples. Farrell (1983) reported that the AME of 47 Australian triticale samples ranged from 13.6 to 16.62 MJ/kg DM in adult cockerels, but a narrower range was reported by Johnson and Eason (1988) who observed values of 12.8 to 14.3 MJ/kg DM in eight samples of triticale. In addition, Vieira *et al.* (1995) reported that the AME of triticale was different between male and female broiler chickens. These authors observed that AMEn values for triticale in male and female broiler chickens were 13.70 and 13.47 MJ/kg, respectively. In comparison with wheat, there was no significant difference in apparent, true, and N-corrected ME values (Salmon, 1984; Boldaji *et al.*, 1986), but triticale values are lower than those of maize (Shimada and Cline, 1974; Halvorson *et al.*, 1983). Maurice *et al.* (1989) reported that the fat content of triticale was only 50% of that in maize which may partially account for the lower ME of triticale. An even narrower range than was found in previous studies was reported by Hughes and Choct (1999) who evaluated three Australian triticale varieties and found that, in broiler chickens, AME values ranged from 12.7 to 13.9 MJ/kg DM. In a later study conducted on six Australian triticale varieties, grown in two different locations, in drought and unstressed conditions, Hughes and Cooper (2002) observed that the AME in broiler chickens varied from 13.6 to 14.1 MJ/kg DM (triticale grown in drought conditions) to 13.9-14.5 MJ/kg (higher rainfall condition).

Wilson and McNab (1975) reported that the starch content of triticale is potentially higher than that of wheat. However, there is a difference of starch content between ‘old’ and ‘new’ triticale cultivars, with the ‘new’ triticale cultivars being higher in starch content. Flores *et al.* (1994b) studied on 18 triticale varieties which showed the starch contents ranging from 563 to 629 g/kg DM, while later studies reported that the starch concentrations varied between 660 and 730 g/kg DM (Leon *et al.*, 1996; Çiftci *et al.*, 2003; Pejin *et al.*, 2009). Moreover, van Barneveld (2002) reported that the total starch concentration of four Australian triticale varieties varied between 602 and 675 g/kg DM. A study was conducted in Czech Republic on three winter varieties of triticale in 2006 and 2007. The total starch of the grains ranged from 624 to 709 g/kg DM (Buresova *et al.*, 2010). In contrast with the study conducted in Ireland, the starch content of Czech triticale were significantly affected by the cultivar and year harvested. Stacey *et al.* (2006) reported that the starch concentrations of Irish triticale cultivars varied between

662 to 675 g/kg DM and its concentrations were not significantly dependent on the harvest time.

Flores *et al.* (1994b) reported that generally starch digestibility in cereal grains was high, (i.e. 96.5-99.4%). The composition of the starch present in the grain (e.g. amylose to amylopectin ratio) influences the digestibility of the grain. The amylose content of triticale seems to be fairly similar to that of wheat. An early report of amylose content in starch in two varieties of American triticale was only 182 and 200 g/kg (Vohra and Shariff (1980). Sharma *et al.* (2002) observed a range of 200 to 300 g/kg in triticale varieties grown in Australia. In comparison, the amylose content in Australian hexaploid wheat cultivars was found to range from 235 to 389 g/kg (Regina, 2000). van Barneveld (2002) reported the amylose content of four Australian triticale varieties varied between 263 and 296 g/kg and Dennett *et al.* (2009) who studied the selection of 247 triticale lines from CIMMYT found that the amylose content varied from 128 to 351 g/kg. The higher amylose ratios reported in these later studies might reflect the progress being made in the development of triticale varieties by the breeders.

Crude protein

Studies on CP content have shown that triticale protein is higher than that of wheat (Varughese *et al.*, 1997). Villegas *et al.* (1970) revealed that the protein content of triticale is about 20 to 30% higher than that of wheat, possibly because of the rye parent. Several reports indicated that the CP content of triticale grain varies between 90 and 200 g/kg DM (Johnson and Eason, 1988; Heger and Eggum, 1991; Metayer *et al.*, 1993; Flores *et al.*, 1994b; Vieira *et al.*, 1995; Boros, 1999; Erekul and Köhn, 2006). In addition, the protein digestibility of triticale grain is high, compared to that of wheat, with values ranging from 79.2 to 81.9% (Elangovan *et al.*, 2011).

Amino acids

Protein quality in cereals is largely dependent upon available lysine content, as this amino acid is usually the first limiting in cereal grain protein (Silano, 1977), but in some triticale genotypes, lysine content is much higher than in wheat or rye (Ruckman *et al.*, 1973; Salmon, 1984; Gatel *et al.*, 1985; Stallknecht *et al.*, 1996; O'Brien, 1999). Furthermore, Salmon (1984) found that arginine, aspartic acid and alanine concentrations were also higher in triticale than wheat. The concentrations of threonine and sulphur amino acids were similar to those of wheat, but triticale was poorer in tryptophan (Gatel *et al.*, 1985). Ruckman *et al.* (1973) also reported

that protein and lysine contents were remarkably constant in similar varieties of triticale harvested in different years and locations. The amino acid contents appeared to be similar although the grains were harvested at different locations (i.e. Spain and Australia) (Flores *et al.*, 1994b; van Barneveld, 2002).

In addition, Flores *et al.* (1994b) reported that there were no differences in digestibilities of specific amino acids of 18 triticale varieties grown in Spain. The higher levels of lysine (or other amino acids) in triticale have not been linked with increases in animal performance (Mosse *et al.*, 1988). This is probably because modern dietary formulations make allowance for different amino acid concentrations and availabilities of individual dietary ingredients.

The concentrations of amino acids in various triticale varieties as well as apparent ileal digestibility for broiler chickens are presented in Table 2.1.

Table 2.1 Amino acid content and digestibility in triticale varieties

Amino acids	Min-Max (Mean ± SD)[1] (N =18 varieties) (g/kg DM)	Min-Max (Mean ± SD)[2] (N = 4 varieties) (g/kg DM)	Coefficient of apparent ileal digestibility[3]
Aspartic acid	7.4-10.0	7.0-8.0	0.83
Threonine	4.0-6.3	3.6-4.2	0.78
Serine	6.1-8.2	5.0-5.9	0.83
Glutamic acid	25.5-36.1	24.4-30.2	0.94
Proline	10.1-15.0	8.2-11.3	0.92
Glycine	4.2-6.6	4.7-5.4	0.80
Alanine	7.3-9.4	4.6-5.4	0.84
Valine	5.6-8.0	4.7-6.4	0.87
Methionine	nd	1.9-2.3	0.88
Isoleucine	3.4-5.3	3.2-4.7	0.88
Leucine	7.8-10.4	6.6-8.1	0.89
Tyrosine	3.4-5.1	3.1-3.5	0.74
Phenylalanine	5.0-6.9	4.2-5.6	0.86
Lysine	3.3-6.2	3.7-4.4	0.89
Histidine	2.7-3.9	2.7-3.4	0.86
Arginine	3.6-13.8	5.2-6.7	0.87
Cysteine	nd	2.3-2.8	0.77
Tryptophan	nd	1.0-1.3	nd

[1]Flores et al. (1994b); [2]van Barneveld (2002); [3]Perttila *et al.* (2005); nd = not determined

Minerals

The variations in mineral concentration of triticale are due to factors such as soil type, irrigation, fertiliser, seasonal conditions of growth and variety (Singh and Dodda, 1979); however, in comparisons between triticale and wheat, triticale contained higher amounts of Ca, K, P and Mg (Lorenz *et al.*, 1974; Sehgal *et al.*, 1983).

Vitamins

The vitamin contents of the whole grain of triticale and wheat when grown under the same conditions have been reported by Michela and Lorenz (1976). The authors found that triticale critically had lower amounts of niacin; but in general the vitamin composition of triticale is similar to that of wheat and better than that of rye. Biotin, folacin and B_6 vitamins were slightly higher in triticale than in wheat, whereas thiamine, riboflavin and pantothenic acid were marginally lower than in wheat.

The mineral compositions of triticale and wheat grains are presented in Table 2.2.

Table 2.2 Mineral concentrations of triticale and wheat grains (dry basis)

Element	Wheat[1] (N = 3 varieties.)	Triticale[1] (N = 5 varieties)	Wheat[2] (N = 2 varieties.)	Triticale[2] (N = 14 varieties)
Ash (%)	Nd	1.8-2.3	1.5-1.6	1.3-2.3
K (mg/g)	4.1-4.9	3.6-5.1	3.3-3.7	4.3-4.9
Mg (mg/g)	1.6-1.9	1.8-2.0	nd	nd
Ca (mg/g)	0.3-0.4	0.3-0.4	0.49-0.52	0.4-0.6
P (mg/g)	3.4-3.7	4.4-5.3	1.8-2.4	2.1-3.2
Na (µg/g)	30.0-50.0	29.0-57.0	213-225	138-5.7
Zn (µg/g)	5.0-30.0	18.0-35.9	41.0-45.0	32.0-56.0
Cu (µg/g)	4.6-5.1	3.2-13.8	6.0-8.0	6.0-10.0
Fe (µg/g)	37.0-44.0	44.7-57.0	83.0-92.0	58.0-110.0
Mn (µg/g)	32.0-38.0	48.6-63.2	35.0-38.0	28.0-39.0

[1]Lorenz et al. (1974); [2]Sehgal et al. (1983); nd = not determined

2.5.3 Limitations to use of triticale

The nutrient profiles discussed in previous sections indicate that triticale has considerable potential as a feed grain with its high ME and protein contents as well as slightly better amino

acid composition than wheat, particularly in relation to lysine. However, toxic factors and nutritional inhibitors have been reported in triticale, which may limit its usefulness in poultry diets. Shimada *et al.* (1974) reported that a triticale basal diet was contaminated by 0.16% ergot, which caused low performance of chickens. Ergot consists of various species of fungi derived from the genus *Claviceps* that are capable of producing mycotoxins in cereals (Langdon, 1950). Similar to the result of Shimada *et al.*, Bragg and Sharby (1970) reported that ergot reduced the weight gains of triticale-soybean meal fed chicks only when the fungus was present at levels higher than 0.80%, meanwhile McCloy *et al.* (1971) reported that ergot is more severe in older than in 'newer' cultivars of triticale.

Alkyl resorcinols and trypsin inhibitor are also implicated as antinutritional factors in triticale. Radcliffe *et al.* (1981) reported that breeding lines of triticale in South Australia have been found to contain 0.06-0.17% of alkyl resorcinol. Trypsin inhibitors have also been found in some Australian triticale lines at levels of activities similar to those in rye (Radcliffe, 1980). Smith *et al.* (1989) reported the growth inhibition in chickens fed triticale-based diets may have been due to the presence of trypsin inhibitors, alkyl resorcinols and pectins. However, Myer and Barnett (1985) reported that trypsin inhibitor activity of 'new' triticale cultivars is less likely to limit the use of triticale in animal feed.

With the 'new cultivars' of triticale, the concern is far more on soluble NSP and phytate as antinutritional factors for poultry. The arabinoxylans and β-glucans present in the endosperm of the cell wall, which are related to the level of NSP of the grain, have been identified as major causes of poor growth rate, low nutrient digestibility as well as less ME in broiler chickens (Pettersson and Åman, 1989; Flores *et al.*, 1994a). The use of triticale in broiler feeds is limited by the presence of soluble NSP, especially arabinoxylans (Salmon, 1984; Rundgren, 1988). Choct (1997) indicated that maize and sorghum contain low levels of NSP, whereas wheat, rye and triticale contain substantial amounts of both soluble and insoluble NSP. The main soluble NSP in these grains are arabinoxylans, whereas barley and oats have β-glucans. Choct and Annison (1990) reported that the higher soluble NSP will reduce the AME of cereal grains in broiler chickens (See Figure 2.4).

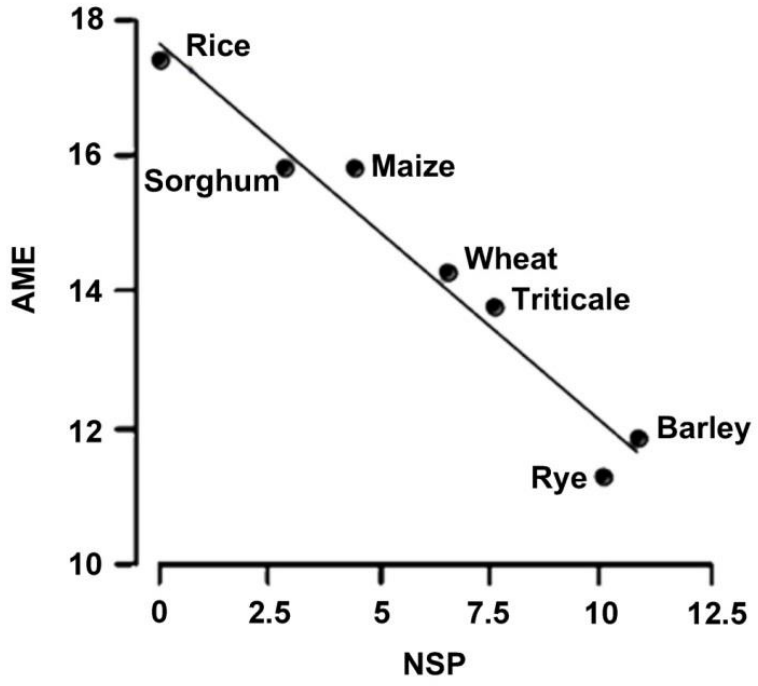

Figure 2.4 The relationship between AME (MJ/kg DM) and the soluble NSP content (% of DM) of cereal grains in broiler chickens (Choct, 1997).

Analysis of two cultivars of triticale (i.e. Carman and Welsh cultivars) by Al-Athari and Guenter (1989) suggested that the pentosan (arabinoxylans) content of Carman triticale (5.73-5.89%) was much lower than that of the earlier Welsh triticale (7.34-8.41%), but closer to that of wheat (4.3%). However, both triticale cultivars were lower in pentosan content than the 9.8% reported for rye (Antoniou *et al.*, 1981). As a result, the higher amounts of pentosans in triticale based diets will increase the gut viscosity, reduce the digestibility of the feed and reduce the performance of broiler chickens (Choct and Annison, 1990).

Phytic acid in triticale is another antinutritional factor that could limit the use of triticale in poultry diet. In general, the percentage of phytate-P varies from 64 to 85% of the total P in most cereals (Ravindran *et al.*, 1994); for triticale, the total P is about 0.37 ± 0.02% and the phytate-P content is 0.25 ± 0.02%, or 67 ± 3.7% phytate-P in total P (Eeckhout and De Paepe, 1994).

2.5.4 Replacement value of triticale in poultry diets

There have been numerous studies to demonstrate the potential role of triticale, especially recently developed cultivars, as a substitute for wheat and maize. However, there is apparently

variation in the nutrient composition of the different varieties of triticale (Bushuk and Lartner, 1980). As has been previously discussed, triticale possesses nutritional qualities similar to those of wheat, although it is higher in lysine content. Triticale also has a good protein and amino acid digestibility, which make it suitable as a substitute for most cereal grains in poultry diet. On the other hand, it appears to have higher concentrations of some antinutritional factors.

In an early study, Sell *et al.* (1962) replaced wheat with 300, 450, 600 or 670 g triticale/kg. They found that the birds grew equally well on all diets but feed conversion efficiency fell when triticale formed 600 g/kg of the grain in the diet. However, these diets were not isonitrogenous because the protein content of triticale was greater than that of the wheat. Bragg and Sharby (1970) concluded from three feeding trials that triticale can completely replace wheat and can constitute up to 660 g per kg of diet with no adverse effects on growth or feed conversion ratio (FCR) of broiler chickens. In addition, these authors concluded that the ME content of triticale was similar to that of wheat. Reddy *et al.* (1979) demonstrated that ME of triticale diet for broiler chickens was slightly higher than that of a wheat diet (11.86 vs 11.64 MJ/kg), but less than a maize diet (12.97 MJ/kg). The potential role for inclusion of triticale as a substitute for maize in broiler and laying hens diets without any detrimental effect on feed intake, digestibility and performance has been demonstrated by other researchers. For example, Rao *et al.* (1976) showed that the replacement of up to 75% of maize with triticale in a broiler chicken diet had no adverse effect on weight gain or FCR, while Vieira *et al.* (1995) demonstrated that the replacement of maize by up to 80% in a maize-soybean meal diet did not influence the bird's overall performance. These studies demonstrate that triticale can replace maize in broiler chicken diets and produce similar levels of performance.

Using 'new' cultivars of triticale in diets for broiler chickens, Zarghi and Golian (2009) observed that, when maize was replaced by up to 75% triticale, the birds had a similar feed intake and similar weight gain to birds on maize diets. However, when these authors totally replaced maize with triticale in diets for broiler chickens, the average daily feed intake and feed conversion efficiency from 4 to 42 days of age were increased. The same authors also reported that exogenous enzyme supplementation increased gizzard and small intestinal weights with an increase in triticale in diet when measured at 18 and 42 days of age. The study revealed that diets containing up to 40% triticale (or 75% of maize replacement) had no negative effect on broiler performance. Similarly, Osek *et al.* (2010) reported that when wheat was partially or completely substituted by triticale, the dressing percentage and muscularity of carcase did not

differ. In addition, feeding triticale alone or in combination with wheat increased the proportion of unsaturated fatty acids in breast muscle lipids, which is a health benefit to the consumer.

With regard to economic efficiency, with 60% replacement of maize by triticale, the cost of feeding was 9.7% lower than that of a maize-based; however poor weight gain, feed intake and FCR were reported on that substitution (Mierliţă, 2008). The author also added that an increase in the viscosity of the intestinal chyme was associated with the triticale diet, which reduced intestinal transit, consequently reduced feed intake and digestibility of the diet. Korver *et al.* (2004) reported that triticale would be an economically feasible replacement for wheat in broiler diets when its price is less or equal to 95% of that of wheat. However the overall FCR, feed cost per kg body weight and total cost of production on the triticale diets were greater than on wheat diets. The use of triticale in broiler chicken diet decreased the bird's body weight by 4 and 8%, when used as a wheat and maize substitute, respectively, in the diet with the whole grain. Similarly, feed conversion per kg of body weight gain also reduced by 8 and 14%, respectively (Osek *et al.*, 2010). Conversely, Santos *et al.* (2008) reported an improved body weight when triticale replaced maize in broiler diet and the Salmonella colonisation was also reduced on the triticale diet. Therefore the authors concluded that triticale is a good alternative to maize.

2.6 IMPROVING THE NUTRITIVE VALUE OF TRITICALE

From the foregoing discussion, it can be deduced that triticale can replace other grains in poultry diets, but despite the improvement made to date through breeding, there are still some factors that could be improved. There are opportunities to improve the nutritive value of triticale, through breeding, feed processing and nutrient and pro-nutrient supplementation.

2.6.1 Breeding

The first triticale breeding programme for spring triticale was held by the International Maize and Wheat Improvement Centre (CIMMYT) and the University of Manitoba in 1965 (Stoskopf, 1985); however, the objective of the programme was to protect the high genetic yield potential of triticale. The genetic and breeding investigations, since then, have compared triticale with other cereals in feeding experiments using different domestic animals as well as investigating a range of nutritional and economic aspects (Gatel *et al.*, 1985; van Barneveld and Cooper, 2002). The main breeding goal was to combine the high content of CP and AME of wheat with

the high protein quality of rye to produce a high-yielding crop usable for animal feeding (Rundgren, 1988).

Triticale was created to combine wheat quality with the winter hardiness and disease resistance of rye as well as to utilise hybrid vigour (NRC, 1989; Varughese *et al.*, 1996). From its parents, triticale possibly has three ploidy levels, viz. octoploid, hexaploid and tetraploid (Boros, 1999). Boros (1999) also noted that the share of R (rye) genome in the total triticale genome pool has a highly significant effect on the nutritional value of triticale. The less the share of R genome in the triticale genome pool, the lower is the antinutritive effect. The lower performance of chicks fed diets based on tetraploid triticale as compared to those fed hexaploid triticales are mainly related to the presence of the R genome. Cyran (1996) reported that a higher share of the R genome in triticale was related to a higher amount of soluble arabinoxylans in triticale.

2.6.2 Feed processing

There are no reports of the effect of feed processing on the nutritive value of triticale for poultry. In general, the purpose of feed processing is to optimise the bioavailability of the nutrients. Chicken diets can be prepared as mash, crumbles and pellets, which differ from one another in particle size. The simplest type of processing is milling, to reduce particle size. After milling, the grain can be subsequently mixed with other feed ingredients and offered to the chickens as a loose mix.

Smaller particles have a smaller surface area, but a much higher surface area to mass ratio, which will allow increased access to digestive enzymes and enhance digestion of nutrients (Waldroup, 1997). Crushing the grain also disrupts the cell wall and endosperm and makes individual components more accessible to digestive enzymes. Lentle *et al.* (2006) suggested that larger particle size is more advantageous in terms of feed efficiency in broiler chickens; however, the particle size effect on bird performance seems to differ, depending upon the type of grain in the diet (Amerah *et al.*, 2007), the type of mill used (Svihus *et al.*, 2004) and whether there is further processing such as pelleting and crumbling (Goodband *et al.*, 2002). Another benefit of larger particle size was reported by Douglas *et al.* (1990) who demonstrated significantly higher body weights and feed conversion efficiency of chickens on diets containing grains ground by a hammer mill compared to those disrupted by a roller mill.

Uniformity of particle size in diets is critical when providing diets for the chicken in mash form to achieve the best performance (Nir *et al.*, 1994). It is also important to produce an optimum

performance of broilers, especially those raised in close confinement using automated feeding equipment in order to produce a consistent size (Waldroup, 1997). McCoy *et al.* (1994) demonstrated that the lower the coefficient of variation or the more homogenous the diet, the higher is the average daily gain and better the feed conversion ratio of growing chicks. Therefore, increased knowledge of particle size implications and diet uniformity is crucial when considering fineness of grind. Yasar (2003) suggested that feeding broiler chickens with finely ground wheat grain in the early growing period is not recommended owing to 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 grains remain the preferred form of cereal grains, although the use of whole grain has the advantage that there is no grinding cost.

Pelleting and crumbling are the alternative feed processing techniques. It has been more than half century since the production benefits of pelleted diets rather than mashes was reported (Hussar and Robblee, 1962). Now, pelleting is the most common form of further processing of poultry diets. Behnke (1994) attributed the benefits of pelleting to: 1) decreased feed wastage; 2) reduced selective feeding; 3) decreased ingredient segregation; 4) less time and energy expended for prehension; 5) destruction of pathogenic organisms; 6) thermal modification of starch and protein; 7) increased digestibility, and 8) improved palatability. Additionally, pelleting improves feed handling characteristics and may reduce feed formulation costs by allowing the use of alternative feed ingredients (Fairfield, 2003). Another benefit of feed processing is reducing the presence or activity of anti-nutritive factors in raw materials. Heat treatment of legumes, for example, reduces the activity of digestive enzyme inhibitors (Gertler and Nitsan, 1970).

In the pelleting process, the conditioning temperature used is the key to optimal bird performance. Moderate heating of broiler diets (80 to 85ºC) seems to improve the nutritional value of the diet; the improvement may be attributed to gelatinisation and increased enzyme susceptibility of starch granules (Silversides and Bedford, 1999a), degradation of heat labile antinutrients (Pickford, 1992), destruction of cell walls and improved availability of nutrients (Cutlip *et al.*, 2008). Silversides and Bedford (1999a) demonstrated that moderate temperatures (80 to 85ºC) resulted in the best performance of broiler chickens. Higher temperatures can cause denaturation of protein (Araba and Dale, 1990), increased content of resistant starch (Blakeney, 1993; Brown, 1996), solubilisation of NSP (Vranješ *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) found that with barley-maize diets, conditioning temperatures as high as 90ºC markedly reduced cellulase activity, energy and nutrient utilisation and as a consequence there was decreased performance of broiler chickens. Other researchers have reported negative effects of pelleting at less than 90ºC. Bedford *et al.* (2003) reported that there was evidence in wheat-based diets that once pelleting temperature exceeded 65ºC (measured when pellets exit the pellet die) both weight gain and feed per gain were reduced. Cowieson *et al.* (2005) showed that increasing conditioning temperatures from 80 to 90ºC reduced weight gain and resulted in a poorer FCR in broiler chickens. In maize-soy-based diets, Kirkpinar and Basmacioğlu (2006) showed that pelleting at 65ºC resulted in higher weight gain compared to a basal mash diet and pelleting at 75 and 85ºC. In addition, Creswell and Bedford (2006) reported that the AME content of maize-based diets pelleted at 86ºC was 3% lower than that of grain pelleted at 69ºC. In summary, the performance of broiler chickens is reduced by the use of temperatures greater than 85ºC during pelleting; however, the optimum temperature probably differs between the grains used in the diet.

2.6.3 Use of supplements

A balanced diet for poultry is one that provides the appropriate quantities of biologically available nutrients required by the bird. Beside digestible energy and protein, essential minerals, vitamins and specific amino acids must be considered (Ravindran, 2011). Triticale has been used as an ingredient for poultry for more than a century. Owing to some limitations of the grain, the use of supplements must be considered in order to improve the nutritive value of triticale and to maximise the efficiency, health and well-being of the bird. The supplements that have been used include enzymes, crystalline amino acids and gut ecosystem enhancers such as probiotics and prebiotics.

A number of exogenous enzyme supplements have been used in poultry diets to improve feed utilisation. These enzymes include carbohydrases (mainly xylanase and β-glucanase), proteases and phytase. Exogenous enzymes are used either to correct the lack of specific endogenous digestive enzymes in the bird or to hydrolyse anti-nutritional factors in feed ingredients (Blakeney, 1993; Bedford and Schulze, 1998; Simon, 1998; Barletta, 2001).

The first application of exogenous enzymes in triticale-based diet was reported by Pettersson and Åman (1988). Consistent with earlier feeding trials in which pentosanase and β-glucanase were included in rye and barley-based diets for broiler chickens, the supplementation of high pentosanase and β-glucanase in triticale-based diets improved growth performance. Pentosans were assumed to be the factors responsible for the low growth performance of broiler chickens given rye-based diets (Antoniou and Marquardt, 1981; Antoniou *et al.*, 1981). The content of pentosans (sum of arabinose and xylose residues) in cereal grains is lowest in wheat and triticale (5.0 and 5.1%, respectively) while rye has the highest content (7.1%) (Pettersson and Åman, 1988). These authors found that enzyme supplementation had little effect on production of birds given diets composed of wheat and two cultivars of triticale (Lasko and WW 31433); however, feed intake, weight gain and feed efficiency were increased in supplemented birds given triticale- (Sv 8008) and rye-based diets. The authors indicated there was a close resemblance between wheat and the triticale cultivar Lasko, while rye and triticale cultivar Sv 8008 were more similar to each other. Triticale cultivar WW 31433 was of an intermediate quality. In 1990, Richter *et al.* (1990a) showed that an application of the proprietary product, Endofeed (β-glucanase and pentosanase), in a diet based on triticale cultivar 'Grado' had no effect on mortality. On the other hand, Flores *et al.* (1994a) found that a commercial enzyme containing β-glucanase, hemicellulase, cellulase and pentosanase activities generated an improvement in the ME and feed efficiency of broiler birds on a diet containing 300 g/kg triticale; however, for birds given a diet containing 600 g/kg triticale, ME did not change, but the weight gain and feed efficiency were improved. The authors concluded that the enzyme supplementation did not correlate with the soluble pentosans of the triticale as has been claimed by different authors (Fengler and Marquardt, 1988; Pettersson and Åman, 1988, 1989). Józefiak *et al.* (2007) reported that the addition of exogenous xylanase to a triticale-based diet resulted in a higher body weight gain of birds than those to rye- and wheat-based diets with the inclusion of exogenous xylanase. The inclusion of xylanase in a triticale-based diet used to feed broiler chickens decreased their ileal digesta viscosity (Józefiak *et al.*, 2004a; Fabijanska *et al.*, 2007), increased the concentration of organic acids in both the ileum and caeca (Józefiak *et al.*, 2004a; Józefiak *et al.*, 2007), and increased the digestibility of amino acids (Im *et al.*, 1999).

Grains or grain products contain 60-80% of their total P in the form of phytic acid (Singh *et al.*, 2003). Triticale grain has a higher phytate-P content and a higher phytate-P to total P ratio than rye, wheat, barley and peas (Eeckhout and De Paepe, 1994). The inclusion of exogenous phytase in poultry diet compensates for a lack endogenous phytase that is required to hydrolyse

the phytate and releases the P in a form than can be absorbed from the gut of the bird (Nelson, 1976). When the availability of P is improved, the availability of Ca is also increased and the amount of P in the droppings is decreased (Simons *et al.*, 1990). Supplementary phytase in triticale-based diets has beneficial effects on broiler performance by significantly increasing feed intake and body weight gain; however there may be no influence on feed conversion ratio and tibia ash content (Pintar *et al.*, 2004). The addition of different levels of microbial phytase to maize-based diets significantly improved apparent availability of P in broiler chickens (Dilger *et al.*, 2004; Martinez-Amezcua *et al.*, 2006).

In triticale, as in most cereal grains, the 'first limiting' amino acid is lysine; however, lysine is present in triticale in higher proportions than in commercial wheat (Brown, 1989). After lysine; methionine, threonine, tryptophan, isoleucine and arginine are the next limiting essential amino acids in most poultry diets (Elliot, 1995). In a study of triticale-based diets for broiler chickens, when an enzyme cocktail was added to the diet, almost all amino acid requirements of broiler chicks were met, except for methionine, lysine, threonine and isoleucine, and in triticale, these amino acids were the 1st, 2nd, 3rd and 4th limiting amino acids, respectively (Attia and Abd-El Rahman, 2001). Therefore, even with the addition of supplementary enzymes to the diet, specific crystalline amino acids also need to be included in the diet to increase the available level of specific amino acids. The supplementation of methionine at 0.6 g/kg in maize and wheat-based diets in which the primary grain was replaced with 10, 20, 30, 40, 50, 60 and 68% triticale, resulted in similar feed intakes. However, bird performance was compromised at higher levels of triticale inclusion and the recommendation was for not more than 30% triticale to be included in broiler diets (Richter *et al.*, 1990b). The nutritive value of the diet was lower with the higher level of triticale, which may be the result of anti-nutrient factors in the triticale grain, or amino acid imbalance in those diets. In broiler chickens, an over-supply of individual amino acids may lead to depressed performance, and inefficient and uneconomical meat production (Waldroup *et al.*, 1976).

Recently, the prophylactic use of antibiotics as growth promoters was prohibited in some countries, and in other countries, a number of in-feed antibiotics have also been restricted for use in poultry diets. There is therefore a need to find potential alternatives for antibiotics. The roles of these alternative growth promotants include changing GIT pH, maintenance of protective intestinal mucins, stimulation of the growth of beneficial intestinal microorganisms, enhanced nutrient uptake, and increased humoral immune response (Ferket, 2003). Results of

some studies on antibiotic replacers, such as probiotics and prebiotics, have demonstrated positive results in weight gain, feed conversion and the health of birds (Khaksefidi and Rahimi, 2000; Cmiljanic *et al.*, 2001). A variety of microbial species have been used as probiotics, including species of *Bacillus, Bifidobacterium, Enterococcus, Escherichia coli, Lactobacillus, Lactococcus, Streptococcus*, a variety of yeast species, and undefined mixed cultures. *Lactobacillus and Bifidobacterium* species have been used most extensively in human nutrition, whereas in poultry, these products are based on naturally occurring bacterial genus or species generally recognised as not being of risk to the bird (Simon *et al.*, 2001). The advantages to the bird of supplementing these microbes include promotion of a healthy intestinal balance, enhancement of the mucosal barrier and immune response and establishment of a 'good' microbial population in the gut that can out-compete 'bad' bacteria (such as *Salmonella enteritidis*) in the tract by competitive exclusion (Nuotio *et al.*, 1992). Lan (2004) has argued that prebiotics have a great potential to modulate colonic microflora and discourage the colonisation of enteric pathogens. Any ingredient that enters the large intestine is a potential prebiotic but to be an effective prebiotic; it must be fermented by microorganisms that benefit the host. In the broiler chicken diet, providing low-molecular weight carbohydrates can be favourable to bird health and performance. Dietary mannan-oligosaccharides, for instance, have been shown to simulate the functions of mannan-bearing glycoproteins found on the gut epithelial surface, which serve as attachment sites for bacteria that possess type-1 fimbriae (mannose-binding cell surface lectins), including pathogenic strains like *E. coli* and *Salmonella spp.* (Oyofo *et al.*, 1989). Iji and Tivey (1998) explained how the use of natural or synthetic oligosaccharides in broiler chicken diets can stimulate the blood immune system, promote the carbohydrate fermentation for production of bacteriostatic short chain fatty acids (SCFA), and provide an alternative binding site for bacterial surface receptors responsible for attachment to the epithelium to facilitate colonisation. Prebiotics, also assist the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the GIT (Gibson and Roberfroid, 1995). These are mainly those that produce SCFA.

2.7 IMPORTANCE OF CURRENT STUDY

It is clear from published studies that high-yielding triticale cultivars have the potential to replace wheat and other cereals in diets for non-ruminant animals, especially poultry. High-yielding triticale cultivars, which are generally classified as 'new' triticale varieties, are different from the early triticale varieties, which have a poor feeding value compared to wheat.

New varieties not only have a higher yield than wheat or other cereals but are more robust and more attractive agronomically. They may have higher nutritive value for poultry, especially for broiler chickens than conventional grains such as maize and wheat. Triticale is also a suitable crop for cultivation in some temperate climatic zones of Australia. At extreme locations, such as those subject to drought and acid soils, triticale is more productive than wheat. In Australia, the use of triticale in human food is negligible; therefore, it is cheaper and more attractive as an animal feed resource than wheat.

Since 1979, triticale-breeding programmes in Australia have been undertaken at the University of Adelaide, University of Sydney and UNE, and a number of new cultivars have been released. The breeding group at UNE has developed varieties that are more high-yielding and more disease-resistant than the current commercial strains. Although these new cultivars are promising, a large variation in nutrient composition between cultivars still exists. As can be seen from the literature covered, the existing cultivars exhibit varying levels of ME for broiler chickens and differences in CP digestibility, better lysine and essential amino acid composition but more NSP and phytate than the 'old' cultivars.

Ten new varieties of high-yielding triticale were harvested in two harvest years (2008, which was dry and 2009, which was wet, during the growing season). These differences in conditions provided an opportunity to assess the effect of environmental conditions on quality and nutritive value of triticale. In particular, there is a need to determine the value of triticale as a replacement for wheat and maize in broiler chicken diets. The central aim of the work undertaken and included in this thesis was to investigate the replacement value of the 'new' varieties of triticale when substituted for wheat and maize in diets for broiler chickens. In order to increase the triticale replacement value, the use of key supplementary ingredient exogenous enzymes, was investigated.

The physicochemical composition of the new triticale varieties are addressed in Chapter 3. Energy and nutrient utilisation as well as replacement value of triticale to maize and wheat are covered in Chapters 4 and 5. The influence of microbial enzymes on the nutritive value and digestive enzyme activities are assessed in Chapters 6 and 7, with Chapter 7 focussing more on changes in gut microbial profiles and their activities. It is expected that the findings from this project will further assist the breeding group in their work. Most importantly, it will position triticale as an energy feedstuff for poultry.

CHAPTER 3 NUTRITIONAL CHARACTERISTICS OF NEW CULTIVARS OF HIGH-YIELDING TRITICALE

3.1 INTRODUCTION

Triticale (*X Triticosecale* Wittmack) is the first agricultural crop produced scientifically by humans since the late 19th century. It is the product of a cross between wheat (*Triticum*) and rye (*Secale*). It has been primarily developed as a feed grain for livestock owing to its low versatility for the human food market compared to the other conventional cereal grains such as wheat (King, 2011). However, the world production of triticale has more than doubled in recent years, from 6.4 million tonnes in 2006 to 13.7 million tonnes in 2009 (FAO, 2010). Besides that, in Australia, although it is a minor cereal grain compared to wheat, barley, sorghum and maize, for the last 5 years, the domestic use of this cereal for animal feed has increased, e.g. from 202,200 tonnes in 2006 to 788,000 tonnes in 2010 (To *et al.*, 2010).

Since the first commercial triticale cultivars were released in 1969, many studies have been carried out associated with the development of triticale. Although triticale cultivars demonstrate many agronomic advantages including winter hardiness, drought and disease tolerance (Boros, 1999), excellent productivity potential (Gatel *et al.*, 1985; Vohra *et al.*, 1991) and greater flexibility to adapt to difficult agronomic conditions than wheat (Korver *et al.*, 2004), they have been found to exhibit a wide variation in nutrient content between the cultivars (Vieira *et al.*, 1995).

Over the past 25 years, in Australia, the breeding and selection programs have been active and are still being undertaken, in New South Wales, South Australia and Western Australia, to improve the quality and yield of triticale (Andrews *et al.*, 1991). A crop breeding group at UNE has developed varieties that are even more high-yielding and more disease-resistant and tolerant of acid soil than the current commercial strains, as well as being high in energy content (UNE, 2008). This group has bred cultivars, which have broken the 10-tonne-per-hectare barrier for the yield of a wheat-related grain crop. In addition, for comparison, Australia's average wheat yield is only 2 tonnes/hectare, while in Europe; wheat yields do not exceed 6 to 8 tonnes/hectare (Scanlan, 2005). These varieties hold much promise for the poultry industry, which could use this grain as an alternative energy source. However, there have been no studies of their nutritional quality, and no feeding trials with diets based on these varieties to ascertain their nutritive value. This study was therefore conducted to evaluate the variation in nutritional

characteristics of high-yielding cultivars produced by UNE's crop breeding group. The samples were subjected to proximate analysis and detailed analyses.

3.2 MATERIALS AND METHODS

A total of 10 triticale cultivars obtained from UNE's crop breeding group, harvested in 2008 and 2009 were investigated. According to the breeding group, there were marked differences in harvest conditions between the two years, 2009 being wetter than 2008. The 10 high-yielding triticale cultivars tested were AT528, H20, H127, H128, H157, H249, H418, H426, JRCT74 and Tahara.

The grain samples were ground to pass through a 1 mm sieve for laboratory analyses, while for the starch granule analysis, a sub-sample of whole grains was collected and stored in 20 mL container. The analyses of the samples are described below.

3.2.1 Dry matter and moisture contents

The dry matter (DM) and moisture content were conducted by the following methods. Approximately 2 g were weighed in duplicate into cooled 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 105ºC. Samples were set at this temperature for 24 h and then cooled in the desiccator for 40 to 45 min to a constant weight. DM and moisture contents were calculated based on the loss in weight of the sample following drying.

3.2.2 Crude protein

The nitrogen content of the samples was determined according to the Dumas combustion technique: AOAC official method 990.03, AOAC (2002) using a LECO®FP2000 automatic nitrogen analyser (LECO Corporation, St. Joseph, MI, USA). In order to interpret the detector response as percentage N (w/w), calibration was carried out using a primary standard of pure ethylenediaminetetraacetic acid (EDTA). And a multiplication factor of 5.76 was used to calculate CP from the total N (McKenzie and Farrell, 1980).

3.2.3 Crude fat

Crude fat or ether extract (EE) content was determined by the Soxhlet apparatus. Fat in triticale cultivars samples was extracted by a solvent and the weight of the recovered fat was

determined. Two gram of sub-sample was weighed into a pre-weighed dry porous thimble and sealed with a wad of cotton. This was placed in the oven at 105ºC for 24 h to obtain the dry sample weight. Dried samples were then extracted for 24 h with chloroform as a solvent. The thimble with wad of cotton and samples were allowed to drain and then dried at 105ºC for 24 h. Crude fat was determined as loss in weight of the original sample following extraction and drying.

3.2.4 Ash analysis

For determination of crude ash, 2 g of ground sample were weighed in duplicate into pre-weighed silica crucibles. The samples were dried at 105ºC for about 24 h, and the dried weight was recorded. The dried samples were then ignited in a Carbolite CWF Furnace 1200 chamber furnace (Carbolite, Sheffield, UK) at 600ºC, held for 2 h and then dried at 105ºC for 1 h. The dry samples were colled in a desiccator for about 40 min and the weight of residue (crude ash) was then recorded.

3.2.5 Gross energy

The gross energy (GE) concentration was determined for individual samples using IKA® WERKE bomb calorimeter (C 7000, GMBH & Co., Staufen, Germany). The chamber was calibrated for the heat of combustion of 0.5 g of benzoic acid (26.457 kJ g^{-1}) prior to sample analysis. Approximately 0.5 g of samples were weighed in duplicate and combusted. The GE value was directly obtained digitally as MJ/kg.

3.2.6 Starch content and composition

Total starch and resistant starch in the samples were determined with the "Megazyme" total starch kit, using the enzyme procedure developed by McCleary *et al.* (1994). Finely ground samples (0.5 mm) were accurately weighed into screw-capped reaction tubes (25 mL) and made wet with 200 µL 80% ethanol. Two mL of dimethyl sulfoxide (DMSO) were added. A further 3 mL of thermo stable α-amylase (3000 U/mL; 45 U/mg at pH 6.0, Megazyme) in 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (50 mM, pH 7.0) were added followed by 0.1 mL amyloglucosidase (3300 U/mL on soluble starch at 4.5, Megazyme) and incubated at 50ºC for 1 h. Glucose was determined colorimetrically after incubating an aliquot (0.1 mL) with 2.25 mL GOPOD (glucose oxidase/peroxidase) reagent (Megazyme D-Glucose) at 50ºC for 20 min and reading the absorbance at 510 nm against a reagent blank. For resistant starch

(RS) determination, samples were treated with dimethyl sulfoxide at 100ºC followed by enzymatic hydrolysis of starch as described in this section.

3.2.7 Starch granule analysis

The seeds of ten cultivars of high-yielding triticale harvested in 2008 and 2009 were evaluated using scanning electron microscopy (SEM) to examine the starch granules. Between 5 and 10 kernels of grains per cultivar were used. The thin sections were dried overnight at 50ºC, prior to reading. After drying, all the samples were placed in a desiccator for about 1 h. The sections were applied to adhesive metal tape attached to a specimen stud, allowing them to be evenly distributed on the surface of the tape. The samples were then coated with gold for 2 min with NeoCoater (MP-19020NCTR). The scanning electron micrographs were captured using a JEOL/Nikon NeoScope (JCM-5000, Tokyo Japan) scanning electron microscope.

3.2.8 Amylose and amylopectin contents

The amylose and amylopectin contents were 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.*, 1997) and by the colorimetric method of iodine binding with amylose (Chrastil, 1987). Ground samples, 20-25 mg were weighed accurately into screw-capped reaction tubes (30 mL) and made wet with 1 mL DMSO and put on a 100ºC heating block for 20 min. After cooling, 2 mL of 95% (v/v) ethanol were added. A further 4 mL of pure ethanol were added and the contents allowed to stand overnight for starch precipitation. After blowing dry (under N_2), 2 mL of DMSO were added and tubes were put on 100ºC heating block for 15 min. On removal from the heating block, 8 mL of Con A solvent were immediately added and the contents thoroughly mixed (this is Solution A). After centrifuging this solution at 2,000 × **g** for 10 min, 0.4 mL of supernatant was transferred to 2 mL microfuge tube and 0.5 mL of Con A was added and gently mixed by repeated inversion. After allowing the tube to stand for about 1 h, the microfuge tubes were centrifuged at 14,000 × **g** for 10 min. The supernatant, 0.5 mL, was transferred to 20 mL centrifuge tube and 1.5 mL of sodium acetate buffer (100 nM, pH 4.5) were added. After denaturing the Con A, 0.1 mL of amyloglucosidase was added and incubated at 40ºC for 10 min. Glucose was determined colorimetrically after incubating an aliquot (0.01 mL) with 2 mL of GOPOD reagent (Megazyme D-Glucose) at 40ºC for 20 min and reading the absorbance at 510 nm against a reagent blank.

3.2.9 Non-starch polysaccharides

Soluble and insoluble NSP of the ground samples were measured as described by Englyst and Hudson (1993) and Theander and Westerlund (1993). Approximately 190-200 mg of ground sample had the fat content extracted with 10 mL hexane, and the residue was then sonicated for 15 min. The sample tubes were then centrifuged at 2000 × g for 15 min at 20ºC and the supernatant was discarded. Five mL of ethanol (80%) were added, the sample was heated for 10 min at 80ºC and then centrifuged at 2000 × g for 10 min. The supernatant was kept for free sugar analysis. The residue was dried with nitrogen gas at 40ºC, and then starch gelatinisation was completed by adding 10 mL acetate buffer (pH 5.0) and heating the samples in water bath at 100ºC for 30 min. The tubes were removed and 50 µl α-amylase was immediately added and returned to 95ºC water bath for 30 min. The tubes were then transferred to 55ºC heating block and 50 µl amyloglucosidase (AMG) were added. The tubes were incubated overnight (about 16 h) at 55ºC and centrifuged at 2000 × g for 30 min. The supernatant was used to determine soluble NSP content, while the residue was kept for measurement of insoluble NSP content.

The soluble NSP content was determined by washing 4 mL of the supernatant three times with 16 mL of 80% ethanol, with the supernatant discarded each time. The residue was dried under nitrogen gas (40ºC), 1 mL of 2 M trifluoroacetic acid was added with stirring bars, and the samples were then incubated at 12ºC for 1 h. The residue was dried with nitrogen gas, washed with distilled water, and then the constituent sugars were reduced and acetylated. To analyse the insoluble NSP content, the residue kept from the sample preparation was washed in distilled water and then acetone, and dried with nitrogen gas. The residue was then hydrolysed with 1 mL of 12 M H_2SO_4 at 30ºC for 1 h, before the sugars were reduced and acetylated, as per the soluble NSP analysis.

The free sugars were determined using the supernatant kept for this purpose, by hydrolysing with 1 mL of 12 M H_2SO_4 at 30ºC for 2 h (Saeman *et al.*, 1963), then reducing and acetylating the sugar as per the previous two analyses. The released sugars in all three solutions were measured on a gas chromatograph (Hewlett Packard Model 427, Packard Instruments, Sydney, NSW, Australia) after calibration with allose as internal standard.

3.2.10 Amino acids

Quantitative amino acid analysis of the ground samples of triticale cultivars was conducted at the Australian Proteome Analysis Facility Ltd, Macquarie University. About 100 mg of sample

underwent liquid hydrolysis in 6 M HCl at 110ºC for 24 h. After hydrolysis all amino acids were analysed using the Water AccQTag Ultra Chemistry. The samples were analysed in duplicate. However, cysteine and tryptophan were not determined in this study due to the different method was used.

3.2.11 Mineral concentrations

The mineral contents of the ground grain samples were determined by the microwave digestion technique. The samples, 0.45 g, were weighed into a Teflon® TFM vessel. In a fume hood, 8 mL of nitric acid (70% v/v) were added along with 2 mL of hydrogen peroxide (30% v/v). The solution was swirled to homogenise it. The vessel was closed and introduced to the rotor segment, then tightened, using a torque wrench. The segment was inserted into the microwave cavity and the temperature sensor connected. The microwave program was run to completion (took about 45 min) and the rotor cooled by air until the solution reached room temperature. The vessel was opened and the solution quantitatively transferred into a 50-mL volumetric flask. The solution was made to 50 mL total volume with deionised water and mixed well prior to analysis. The subsequent mineral determination was carried out by radial system Varian model VISTA-MPX Simultaneous, Inductively Coupled Plasma-Optical Emission Spectrometer (Varian© Australia Pty Ltd).

3.2.12 Phytate-P content

The phytate-P content of the grain samples was determined using a sensitive method for the rapid determination of phytate-P in cereals and cereal product as described by Haug and Lantzsch (1983). The following three solutions were required for assaying phytate-P: solution 1 was phytate reference solution. This solution was prepared by diluting the stock solution (0.15 g sodium phytate in 100 mL Milli-Q water) with HCl in a range from 3 to 30 µg mL^{-1} phytate phosphorus), solution 2 was ferric solution, made by dissolving 0.2 g ammonium iron sulphate.$12H_2O$ (Merck kGaA) in 100 mL of 2 N HCl and made up to 1000 mL with Milli-Q water, solution 3 was 2,2-bypyridine solution, prepared by dissolving 10 g of 2,2-bypyridine (Merck kGaA, 5427) and 10 mL thioglycolic acid (Merck Art, 700) in Milli-Q water and made up to 1000 mL.

Around 60 mg of finely ground samples were weighed into 16 mL test tubes and 10 mL 0.2 M HCl solution was added. After that, 2 mL of solution 1 was added; the tubes were heated in boiling water for 30 min and allowed to cool down at room temperature. Two mL of solution

3 were added and the contents thoroughly mixed. Phytate-P content was determined colorimetrically at an absorbance of 519 nm against a blank after adding bipyridine solution. The absorbance was measured within 0.5 to 1 min after bypyridine solution (solution 3) was added.

3.2.13 *In vitro* nutrient digestibility

In vitro digestibility was carried out according to the method described by Babinszky *et al.* (1990), with slight modifications. Digestibility was measured for DM, CP and starch. Five hundred mg of ground samples were digested in triplicate in 12.5 mL of 0.1 M HCl containing 4 g/L pepsin (Sigma Chemical, St. Louis. MO, USA) and the sample was incubated at 40ºC for 1.5 h. Then the sample was dissolved in 2 mL of 110 mg $NaHCO_3$ and 12.5 mL of potassium phosphate buffer (pH 6.8) containing 4 g of pancreatin and 4 mL of amylase per litre. The mixture was incubated at 40ºC for 3 h. After the incubation period, 2.5 mL of Na_2CO_3 (100g/L) were added to each tube and the contents were centrifuged at 3500 × **g** for 15 min. The supernatant was collected and kept on ice until the analysis and the residue was repeatedly rinsed with Milli-Q water. The residue then was freeze-dried and used for determination of DM, CP and starch. Starch was calculated by converting from glucose content. The digestibility of DM, CP and starch was calculated by the following equation:

$$Digestibility\ (\%) = \frac{Weight\ of\ samples\ or\ nutrient\ (mg) - Weight\ of\ dried\ residue\ or\ residual\ nutrient\ (mg)}{Weight\ of\ DM\ or\ nutrient\ in\ samples\ (mg)}\ x\ 100$$

3.2.14 Viscosity

The supernatant collected from the *in vitro* analysis was used to measure viscosity of the grain as described by Bedford and Classen (1993). The Brookfield DVIII viscometer was used, which was set at 25ºC with a cP 40 spindle at 100 rpm. The values were expressed in centipoise (cP), which is 1/100 dyne second per cm^2.

3.2.15 Statistical analysis

A t-test (α <0.05) using 2-sample t in Minitab 16 (Minitab, 2010) was performed to determine whether or not the nutritive value of the cultivars were different between the two different harvest years. If the P-value was less than or equal to the α level, the null hypothesis (H_0) was rejected and it was concluded that there were significant differences in the nutritional value of

the cultivars harvested in 2008 and 2009. The variation in nutritive value within the harvest year was evaluated as coefficient of variation (CV%). A correlation analysis was conducted to determine the relationship between the parameters.

3.3 RESULTS

3.3.1 Proximate composition of triticale cultivars

The proximate composition of different cultivars in the two harvest years is shown in Table 3.1. The DM content varied from 865.5 (H127; harvested in 2009) to 882.7 g/kg (H418; 2009) and the CP content ranged between 92.0 (H128; 2009) and 127.8 g/kg (H418; 2008). The crude fat content varied from 13.9 (H128; 2008) to 27.4 g/kg (Tahara; 2008) while ash concentration was from 16.7 (Tahara; 2008) to 20.0 g/kg (H157; 2009) and gross energy values were between 18.1 (H128; 2009) and 18.5 MJ/kg (Tahara; 2008). The DM, CP, ash content and GE were more uniform (P >0.05), with CV<10%, regardless of harvest year while EE (CV>15%) was more variable.

3.3.2 Starch concentration and composition

Total starch, amylose, amylopectin and resistant starch contents of the ten triticale cultivars are presented in Table 3.2. The starch content ranged from 577.6 (H157; 2008) to 661.6 g/kg (H127; 2008). The values of amylose ranged from 137.9 (Tahara; 2009) to 180.4 g/kg (H249; 2008), whereas the amylopectin content varied from 429.1 (H157; 2008) to 499.8 g/kg (H128; 2009). In percentage terms, amylose was 23.2 to 27.8%, while amylopectin varied between 72.2 and 76.8%. The resistant starch values were between 41.8 (JRCT; 2008) and 50.9 g/kg (H128; 2009). In addition, the amylose:amylopectin ratio ranged from 0.30 (Tahara; 2009) to 0.38 (H20; 2009 and H249; 2008). The total starch, amylose, amylopectin, amylose:amylopectin ratio and resistant starch contents were fairly uniform (CV between 3.65 and 8.47%) and were not different (P >0.05) between the two harvest years.

Table 3.1 Proximate composition (g/kg DM) of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Dry Matter	873.4	874.9	872.6	872.2	873.4	865.5	873.1	876.3	873.3	875.6	873.6	874.9	879.1	882.7	871.2	877.9	873.2	879.1	873.1	878.0	0.24	0.52	0.210
Crude Protein	115.4	106.0	120.8	124.2	115.4	124.4	124.7	92.0	125.3	115.1	118.0	125.1	127.8	114.2	121.7	118.8	122.7	127.8	116.1	108.2	3.67	9.58	0.195
Crude Fat	15.9	20.6	18.4	16.3	15.9	18.8	13.9	20.4	14.6	19.3	13.9	17.5	18.5	17.3	19.1	26.7	19.8	18.5	27.4	23.6	22.72	15.83	0.202
Ash	17.4	18.5	19.8	18.1	19.2	18.3	18.1	19.8	18.7	20.0	17.2	17.5	17.4	17.9	17.5	18.1	19.9	19.2	16.7	18.49	6.22	4.45	0.396
Gross Energy[2]	18.2	18.2	18.3	18.4	18.2	18.2	18.2	18.1	18.4	18.3	18.3	18.4	18.4	18.3	18.4	18.3	18.4	18.4	18.5	18.3	0.50	0.55	0.371

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

Table 3.2 Total starch, amylose, amylopectin, resistant starch contents (g/kg DM) and amylose:amylopectin ratio of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Total Starch	653.7	634.6	622.3	612.3	661.6	653.7	638.9	657.0	577.6	613.6	652.5	606.8	592.3	612.3	626.1	590.0	604.0	612.3	592.3	595.4	4.75	3.65	0.780
Amylose	171.2	155.5	162.1	170.2	168.4	170.1	169.7	157.3	148.5	161.2	180.4	163.1	144.8	159.8	146.5	146.6	150.1	148.1	142.9	137.9	8.47	6.59	0.786
Amylopectin	482.5	479.1	460.2	442.1	493.2	483.5	469.2	499.8	429.1	452.3	472.1	443.7	447.5	452.5	479.6	443.4	453.9	464.2	449.4	457.5	4.16	4.25	0.833
Resistant starch	47.8	48.0	46.1	45.5	48.4	47.2	48.4	50.9	46.3	44.1	49.7	43.8	44.5	45.7	45.3	44.1	41.8	44.2	45.3	47.1	4.97	4.91	0.772
Am:Ap	0.35	0.32	0.35	0.38	0.34	0.35	0.36	0.31	0.35	0.36	0.38	0.37	0.32	0.35	0.31	0.33	0.33	0.32	0.32	0.30	6.66	7.75	0.916

[1]CV = Coefficient of variation; Am:Ap = amylose:amylopectin ratio

3.3.3 Starch granules

The typical micrographs of cultivars of triticale samples harvested in 2008 and 2009 using SEM are shown in Plates 1 and 2.

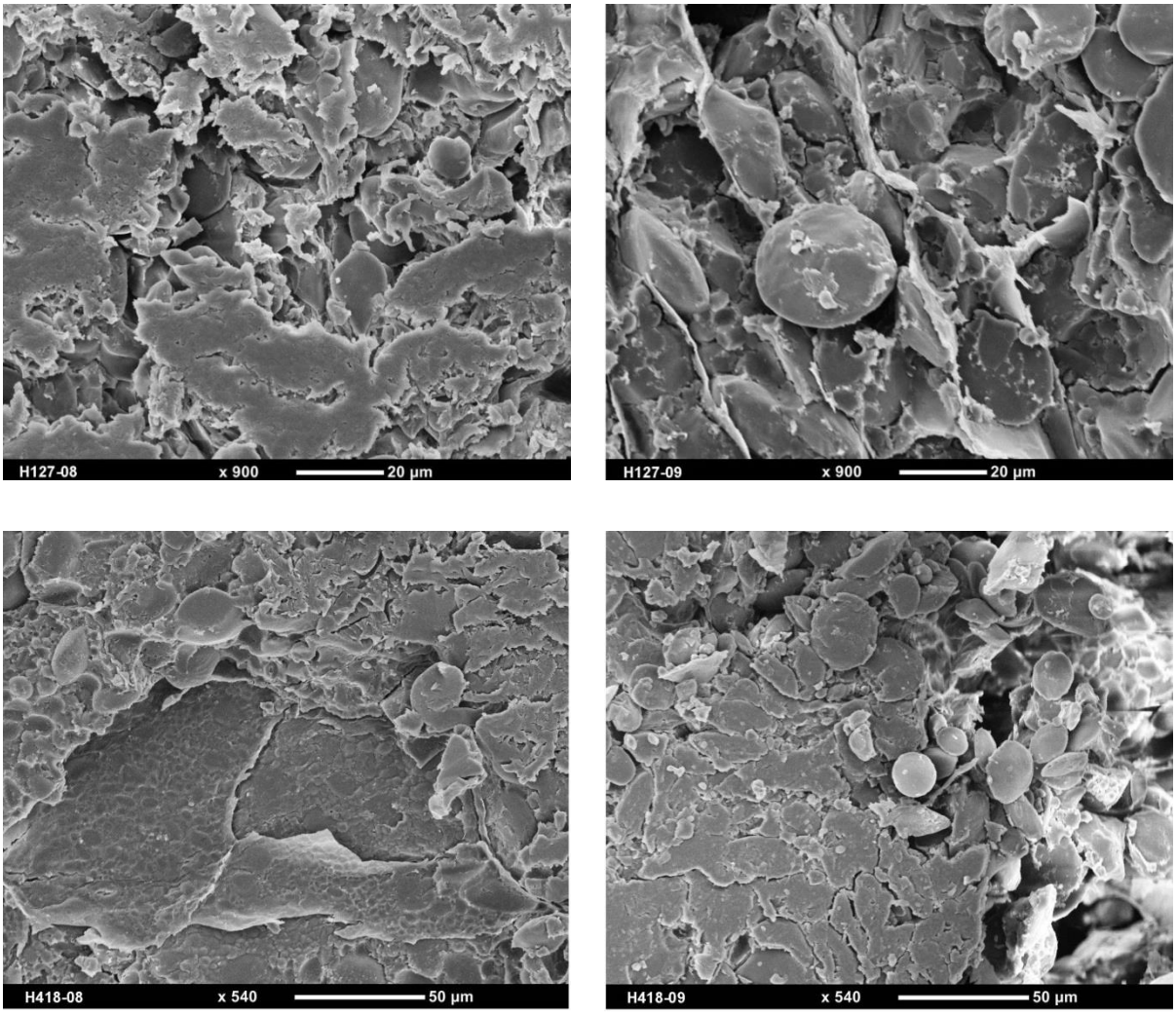

Plate 1: Typical scanning electron micrographs of two cultivars (H127 at top and H418 at bottom) harvested in 2008 (*left panel*) and 2009.

The starch granule shape of all cultivars is round and disk-like, with a pancake appearance. The diameter of the large granules varied between 20 and 30 µm. The small granules and the thickness of the starch granules were varied between 2 and 7 µm in diameter and 5-12 µm in thickness, respectively.

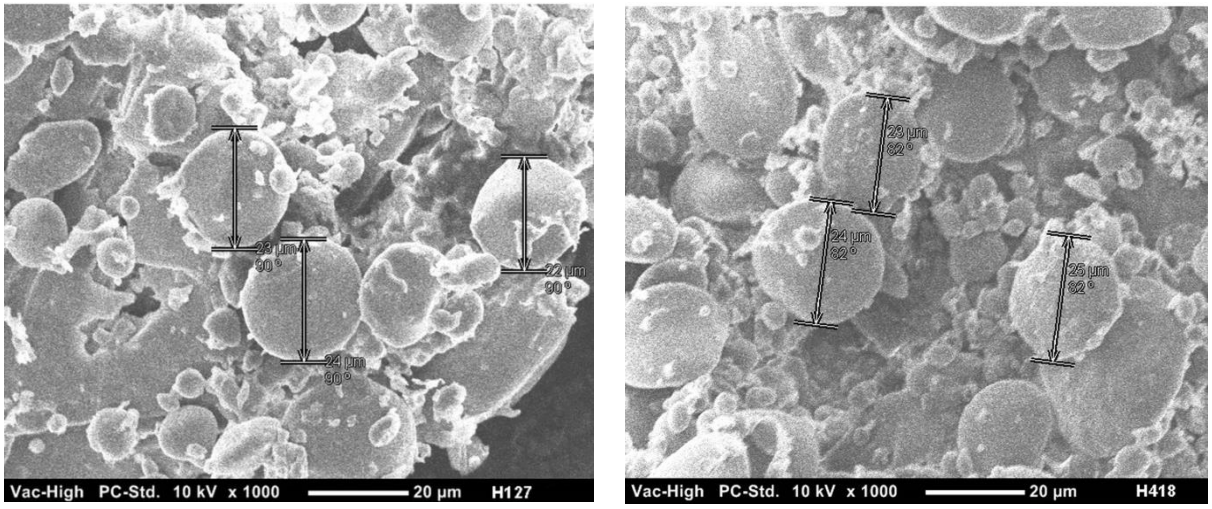

Plate 2: Diameter of typical starch granules from cultivars H127 (*left*) and H418.

3.3.4 Non-starch polysaccharides

The total free sugar values varied from 19.74 (H157; 2009) to 32.75 g/kg DM (H426; 2008) (Table 3.3). Total soluble NSP content ranged between 8.34 (H157; 2009) and 15.09 g/kg DM (AT528; 2008) while insoluble NSP ranged between 79.16 (H20; 2009) and 122.06 g/kg (Tahara; 2009) (Tables 3.4 and 3.5, respectively). The concentrations of the coefficient of the individual free sugars, soluble and insoluble NSP were uniform (P >0.05) between the two harvest years. However, the galactose content of free sugars and mannose component of soluble NSP were different (P <0.05) between cultivars harvested in 2008 and 2009.

3.3.5 Amino acid contents

The amino acid contents of the triticale cultivars are shown in Table 3.6. Nine essential amino acids for poultry (except tryptophan) were analysed. The major essential amino acids in the triticale cultivars were arginine, glycine, valine, leucine and phenylalanine. There were no differences (P >0.05) in amino acid concentrations between the cultivars between the two harvest years as shown by the low CV (from 3.47 to 12.93%).

Table 3.3 Available free sugar contents (g/kg DM) of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Arabinose	0.57	0.47	0.38	0.43	0.58	0.49	0.43	0.61	0.37	0.38	0.43	0.36	0.58	0.45	0.44	0.51	0.46	0.55	0.42	0.53	17.47	15.86	0.76
Xylose	0.21	0.00	0.15	0.19	0.28	0.21	0.22	0.25	0.17	0.00	0.19	0.15	0.29	0.00	0.21	0.19	0.00	0.00	0.00	0.19	58.34	88.59	0.26
Mannose	4.28	4.11	5.65	4.82	3.69	2.99	4.24	3.25	3.58	3.26	4.03	4.12	4.94	2.74	4.74	5.51	4.10	5.25	3.43	4.78	15.94	24.18	0.63
Galactose	1.31	2.00	1.72	2.34	1.47	1.71	1.34	1.91	1.33	1.92	1.53	2.56	1.68	1.75	1.64	2.25	1.19	1.67	1.91	2.26	14.89	14.82	0.00
Glucose	16.61	17.80	18.49	23.23	21.15	15.06	23.13	15.84	17.93	14.18	14.27	19.99	22.85	14.91	25.63	22.22	17.87	20.58	16.45	18.55	18.46	17.63	0.44
Total	23.12	24.39	26.52	31.02	27.17	20.35	29.35	21.73	23.38	19.74	20.48	27.18	30.86	19.86	32.75	30.58	23.62	28.05	22.21	26.37	15.61	17.44	0.60

[1]CV = Coefficient of variation

Table 3.4 Soluble NSP contents (g/kg DM) of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Arabinose	5.22	5.43	4.35	3.40	4.39	3.86	3.55	4.12	3.17	2.91	3.80	2.98	3.04	3.30	2.87	3.43	3.91	3.80	5.12	4.14	20.93	19.64	0.56
Xylose	6.87	6.85	5.73	4.48	5.79	5.00	5.13	5.42	4.32	3.95	4.71	3.70	4.10	4.45	3.63	4.42	4.69	4.93	6.31	5.14	20.12	18.33	0.50
Mannose	1.20	0.47	1.11	1.23	1.06	0.43	0.88	0.48	0.97	0.39	1.10	1.08	1.16	0.38	1.30	0.41	0.39	0.39	0.43	0.47	32.51	54.32	0.01
Galactose	1.28	1.24	1.32	1.16	1.10	1.13	0.91	1.11	1.20	1.19	1.21	1.25	1.01	1.14	1.18	1.24	1.13	1.04	1.27	1.20	10.85	5.72	0.85
Glucose	2.41	1.76	2.51	1.98	1.60	1.70	1.38	1.83	1.23	0.99	1.75	1.35	1.75	2.05	2.23	1.72	1.47	1.60	2.05	1.97	24.09	19.06	0.42
Total	15.09	14.02	13.34	10.86	12.34	10.74	10.49	11.50	9.66	8.34	11.19	9.23	9.82	10.06	9.96	9.98	10.26	10.45	13.46	11.49	16.31	14.32	0.26

[1]CV = Coefficient of variation

Table 3.5 Insoluble NSP contents (g/kg DM) of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Arabinose	30.66	31.03	28.84	23.88	27.62	30.59	29.69	30.64	34.15	41.04	30.73	27.01	29.21	38.14	27.68	34.18	35.88	37.76	38.36	43.10	11.64	18.38	0.30
Xylose	42.29	39.06	38.19	30.86	36.55	38.43	36.58	36.89	44.70	50.03	38.19	32.55	35.99	48.34	36.56	43.67	44.04	47.18	48.50	50.12	10.94	17.15	0.57
Mannose	2.75	4.24	3.38	4.69	4.06	4.63	5.13	4.40	4.30	4.26	2.88	4.16	4.00	4.63	5.14	5.45	4.24	4.29	4.81	4.92	20.95	8.63	0.12
Galactose	2.56	2.26	2.55	2.58	2.77	2.42	3.20	2.33	3.66	2.87	2.93	2.79	3.48	3.44	3.18	2.84	3.21	2.89	3.40	3.57	12.38	15.67	0.13
Glucose	33.64	30.35	27.53	27.35	31.73	26.43	26.27	23.93	36.63	33.44	28.46	31.50	31.35	35.07	28.90	28.62	30.36	30.61	33.86	35.98	10.41	12.70	0.74
Total	99.52	95.43	89.42	79.16	91.27	91.13	89.37	87.27	109.51	116.97	91.80	87.01	92.33	114.93	90.20	102.36	104.65	109.06	114.83	122.06	9.58	14.63	0.56

[1]CV = Coefficient of variation

Table 3.6 Amino acid concentration (g/kg DM) of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Alanine	5.71	5.01	5.62	5.79	5.78	5.70	5.07	4.46	5.58	5.06	5.54	5.95	5.55	5.99	5.27	5.58	5.58	5.95	5.41	5.24	3.84	9.34	0.83
Arginine	7.64	6.63	7.45	7.35	7.73	7.13	6.57	5.41	7.62	6.60	7.36	7.87	7.00	7.59	6.94	7.35	7.51	7.54	7.39	6.96	5.05	10.01	0.29
Aspartic acid	8.84	7.51	7.95	8.37	8.95	8.87	7.74	6.28	8.26	7.31	8.21	8.56	7.80	8.50	7.68	8.11	8.20	8.45	8.06	7.61	5.29	9.82	0.46
Glutamic acid	36.55	30.75	35.49	34.90	37.00	37.76	30.33	24.69	34.66	33.12	36.37	36.91	43.43	37.76	34.97	32.86	34.37	37.53	33.17	28.90	9.41	13.08	0.24
Glycine	6.05	5.72	6.28	6.16	6.12	5.80	5.69	4.58	6.16	5.73	6.26	6.41	6.05	6.34	5.68	5.99	6.16	6.30	6.06	5.87	3.47	8.98	0.39
Histidine	3.47	2.93	3.45	3.37	3.51	3.32	2.91	2.53	3.48	3.06	3.38	3.57	3.56	3.43	3.17	3.36	3.31	3.41	3.24	3.08	5.90	9.63	0.24
Isoleucine	4.81	4.18	4.70	4.74	4.87	4.87	4.12	3.57	4.78	4.28	4.81	5.02	5.03	4.94	4.50	4.62	4.63	4.91	4.52	4.20	5.39	10.21	0.41
Leucine	9.25	8.08	9.03	8.87	9.37	9.28	8.05	6.90	8.98	8.38	9.24	9.49	9.84	9.20	8.71	8.66	8.91	9.15	8.77	7.96	5.25	9.16	0.17
Lysine	4.76	4.29	4.72	4.71	4.82	4.73	4.29	3.81	4.74	4.28	4.60	4.90	4.40	4.60	4.41	4.72	4.62	4.58	4.62	4.76	3.82	6.98	0.45
Methionine	1.82	1.65	1.95	1.95	1.84	1.94	1.63	1.42	1.97	1.46	1.94	1.97	1.84	1.90	1.93	1.72	1.95	1.89	1.80	1.82	5.65	12.93	0.12
Phenylalanine	6.55	5.62	6.33	6.03	6.63	6.46	5.58	4.68	6.44	5.72	6.36	6.54	7.09	6.27	6.14	6.00	6.10	6.23	6.07	6.55	6.36	9.59	0.07
Proline	13.26	11.52	13.39	12.67	13.42	13.10	11.31	9.32	13.10	12.22	13.65	13.72	15.53	13.60	13.10	12.05	12.93	13.51	11.98	13.26	8.34	11.96	0.12
Serine	6.54	5.69	6.58	6.34	6.62	6.38	5.70	4.78	6.51	5.98	6.56	6.67	7.07	6.72	6.18	6.17	6.46	6.68	6.45	6.54	5.36	9.49	0.17
Threonine	4.58	3.93	4.44	4.35	4.64	4.45	3.94	3.39	4.36	3.94	4.42	4.72	4.59	4.39	4.27	4.18	4.40	4.36	4.48	4.58	4.56	8.81	0.10
Tyrosine	3.02	2.54	2.80	2.74	3.05	2.51	2.46	1.92	2.72	2.67	2.94	3.01	2.75	2.81	2.76	2.55	2.86	2.79	2.59	3.02	6.61	11.55	0.08
Valine	6.52	5.58	6.43	6.38	6.60	6.35	5.51	4.88	6.42	5.76	6.42	6.75	6.50	6.52	6.07	6.17	6.31	6.48	6.12	6.52	5.13	9.34	0.29

[1]CV = Coefficient of variation

3.3.6 Minerals

Considerable variation was observed for the mineral contents of triticale cultivars across the two harvest years. The concentrations of Ca, P, K, S, Mg, Na, Fe, Mn and Zn are shown in Table 3.7. The amount of Ca ranged from 0.26 (H426; 2009) to 0.46 g/kg (Tahara; 2008), while the P content varied from 3.50 (H418; 2009) to 4.42 g/kg (H157; 2008). The values for K content were between 4.00 (H418; 2009) and 6.10 g/kg (H157; 2009). Sulphur content varied from 1.18 (H128; 2009) to 1.70 g/kg (H249; 2009) and the Mg content ranged from 1.40 (H127; 2009) to 1.87 g/kg (H418; 2008). The concentrations of Ca, K, S and Mg were similar (P >0.05, CV<30%) between the two harvest years; however P content tended to be different (P =0.007) between 2008 and 2009, but it had a low variance between cultivars each harvest year. For Na, the content ranged from 13.82 (H426; 2008) to 38.79 mg/kg (H249; 2009), whereas Fe content varied from 30.00 (H418; 2009) to 46.33 mg/kg (H426; 2008). The values of Mn were between 38.00 (H127; 2009) and 60.33 mg/kg (H127; 2008) and Zn content was from 20.00 (H127; 2008) to 35.74 mg/kg (H20; 2008). The concentration of Na, Fe, Mn and Zn were not different between the two harvest years or between cultivars in each harvest year.

3.3.7 Phytate-P content

The phytate-P content of the triticale cultivars harvested in 2008 and 2009 are presented in Figures 3.1 and 3.2, respectively. The proportion of phytate-P to total P in the samples harvested in 2008 ranged from 47.2 to 60.4%, whereas for the samples harvested in 2009 it was between 40.8 and 62.6%. Similar to P content, the phytate-P concrentration tended to be different (P=0.08) between the two harvest years, although the variability of phytate-P concentration was relatively low in each year (6.67 and 13.88%, in year 2008 and 2009, respectively). Furthermore the concentration of phytate-P varied between 1.96 (H418) and 2.48 g/kg (H157) in 2008 and 1.80 (H418) and 2.54 g/kg (H157) in 2009.

Table 3.7 Concentration of macro and trace minerals of ten triticale cultivars in two harvest years (DM)

Item		AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Ca		0.37	0.32	0.42	0.40	0.36	0.30	0.37	0.31	0.37	0.36	0.39	0.37	0.40	0.31	0.31	0.26	0.40	0.42	0.46	0.41	10.47	15.54	0.083
P		4.01	3.85	3.98	3.54	4.05	3.70	4.07	3.57	4.42	4.14	3.93	3.59	4.16	3.50	4.02	3.68	4.15	4.12	3.73	3.92	4.38	6.28	0.007
K	g/kg	5.54	5.37	6.06	5.92	5.61	4.70	5.83	5.75	5.67	6.10	5.15	5.41	5.73	4.00	5.71	5.75	5.39	5.74	5.03	5.17	5.55	11.78	0.427
S		1.26	1.46	1.54	1.68	1.45	1.39	1.49	1.18	1.50	1.34	1.53	1.70	1.53	1.43	1.61	1.18	1.57	1.52	1.42	1.33	6.57	12.41	0.296
Mg		1.68	1.68	1.80	1.77	1.67	1.40	1.75	1.44	1.77	1.69	1.63	1.68	1.87	1.55	1.72	1.68	1.77	1.86	1.71	1.73	3.99	8.70	0.099
Na		16.61	18.91	26.42	17.72	26.82	35.00	33.08	26.38	35.04	27.09	24.16	38.79	26.66	21.40	13.82	13.86	24.63	26.50	30.09	21.38	25.61	31.30	0.752
Fe	mg/kg	40.29	42.25	42.33	42.45	40.78	32.00	42.90	30.52	44.36	41.75	39.86	37.64	40.92	30.00	46.33	43.32	39.69	40.67	41.09	42.77	5.12	14.15	0.083
Mn		59.61	53.90	53.49	53.46	60.33	38.00	44.14	38.56	59.69	53.33	47.88	49.35	46.00	42.00	45.90	42.14	51.40	45.72	47.30	52.50	12.29	13.63	0.119
Zn		30.45	27.16	33.34	35.74	30.82	20.00	31.85	25.22	34.86	23.92	30.27	32.32	24.13	30.00	33.75	28.06	28.87	23.98	29.94	29.11	9.80	16.55	0.078

[1]CV = Coefficient of variation

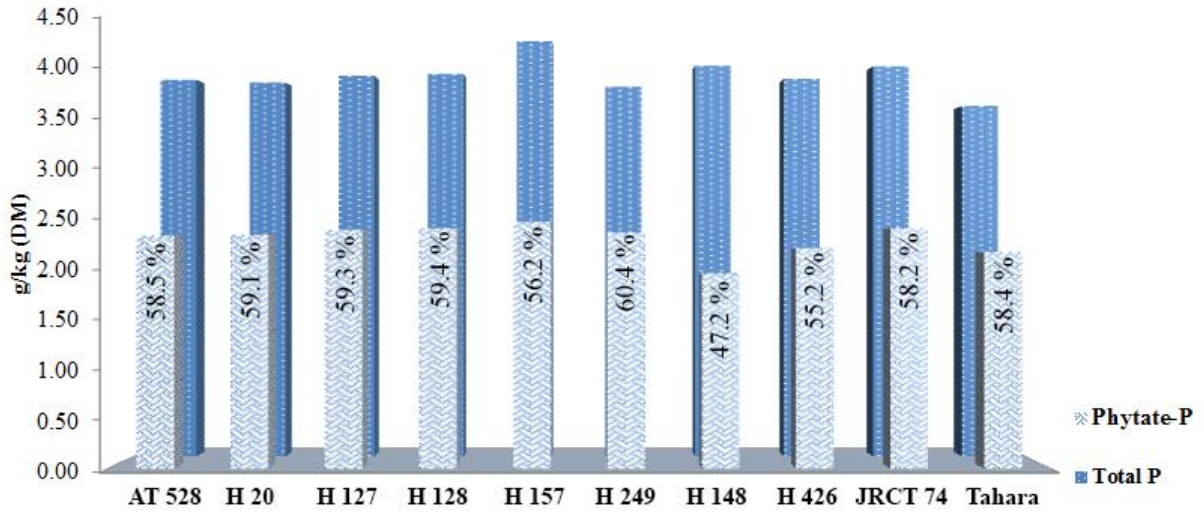

Figure 3.1 Proportion of phytate-P to total P in triticale cultivars harvested in 2008.

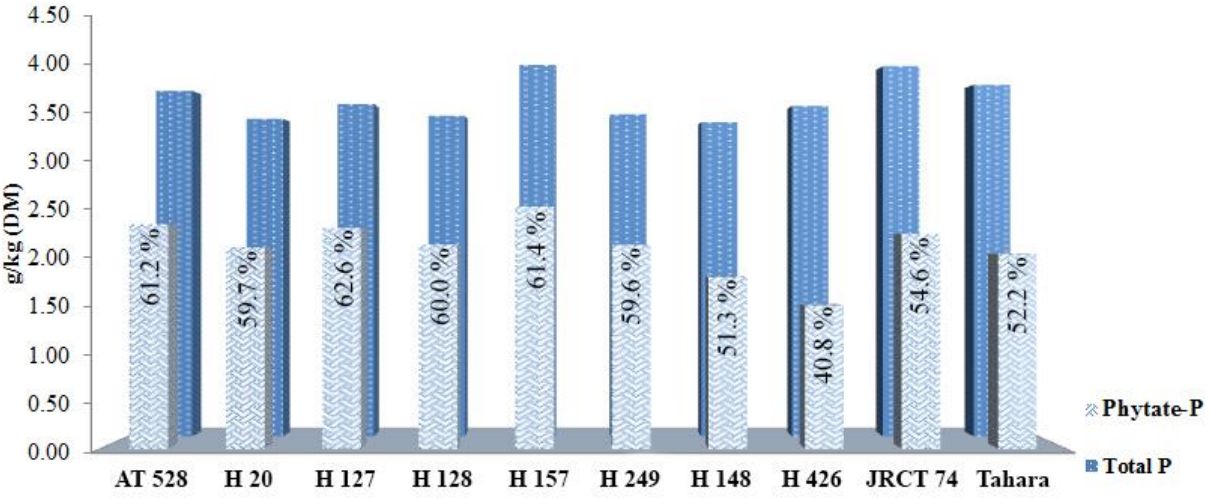

Figure 3.2 Proportion of phytate-P to total P in triticale cultivars harvested in 2009.

3.3.8 *In vitro* digestibility and digesta viscosity

The *In vitro* digestibility and digesta viscosity of the cultivars is presented in Table 3.9.The DM digestibility ranged from 74.01 (H157; 2008) to 77.63% (H418; 2008), while the digestibility of CP ranged from 37.87 (H426; 2008) to 60.72% (H429; 2009) and the starch digestibility ranged from 83.59 (AT528; 2009) to 87.81% (AT528; 2008). The digestibility of DM, CP and starch between 2008 and 2009 were not only different (P >0.05) but also the difference in digestibility between the cultivars was low (CV<15%). In addition, the viscosity during the digestion ranged from 1.01 to 1.09 cP. Viscosity of the same cultivars harvested in 2008 was about the same (P >0.05) as that of samples harvested in 2009, with a CV less than 3% in both years.

Table 3.8 *In vitro* DM, CP and starch digestibility (%) and viscosity (cP)[1] of ten triticale cultivars in two harvest years

Item	AT528		H20		H127		H128		H157		H249		H418		H426		JRCT74		Tahara		CV%[1]		*P*
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	
Dry Matter	76.95	74.20	75.28	75.38	76.30	75.44	77.50	76.79	74.01	76.47	76.47	77.34	77.63	76.23	77.36	74.56	76.86	74.41	75.38	75.48	1.52	1.40	0.15
Crude Protein	57.12	55.85	57.54	52.17	49.38	52.22	53.11	49.98	51.81	39.73	42.15	60.72	48.73	42.70	37.87	43.86	42.67	48.92	40.79	50.46	14.44	12.65	0.61
Starch	83.83	87.80	84.11	83.97	87.18	85.41	85.09	87.51	85.41	84.59	84.61	83.59	84.75	87.43	87.81	86.81	86.54	84.01	85.84	85.24	1.54	1.89	0.86
Viscosity	1.04	1.03	1.09	1.01	1.08	1.04	1.03	1.03	1.02	1.02	1.04	1.09	1.08	1.05	1.02	1.06	1.03	1.04	1.01	1.03	2.75	2.17	0.73

[1]cP (in centipoises) = 1 mPa s (milliPascal second) = 1/100 dyne-second per square centimetre (dyn·s/cm²);
[2]CV = Coefficient of variation

3.4 DISCUSSION

3.4.1 Proximate composition

The nutrient composition of grains is affected by many factors, including grain variety (genetic factors), growing location (agronomic condition) and the season or environmental factors (O'Brien, 1999). However, it is obvious that, in this study of the ten cultivars of triticale, nutrient composition was similar between the cultivars as well as two consecutive harvesting years. Such similarities between grain batches have been reported by Radcliffe *et al.* (1983) who studied nine cultivars of triticale grown at seven different locations in South Australia; Adeola *et al.* (1986) who assessed two cultivars from two different years; Heger and Eggum (1991) who investigated fifteen Czechoslovak cultivars of triticale grown at two locations; Salmon *et al.* (2002) who studied five different cultivars of Canadian triticale, and Brand *et al.* (2003) who reported on ten cultivars at ten different locations in South Africa from three consecutive years. The crop appears to have genetic uniformity between the cultivars, although breeding programmes are still keen to improve their quality.

The DM content of triticale in the current study was less than values reported by Radcliffe *et al.* (1983), Brand *et al.* (2003) and Salmon *et al.* (2002) but this may be the result of differences in varieties as well as the growth location of the triticale used in the studies. The triticale cultivars used in the current study were the spring varieties, popular in Australia (Cooper *et al.*, 2004) whereas the results reported by Heger and Eggum (1991) and Brand *et al.* (2003) were for winter varieties studied in Czechoslovakia and South Africa, respectively, as well as Adeola *et al.* (1986) and Salmon *et al.* (2002) in Canada. Other factors that can possibly affect the nutrient composition are weather, management, postharvest storage, pathogens and pests (Morris, 2004).

The protein content of triticale cultivars in this study was lower than that reported by Brand *et al.* (2003) and Salmon *et al.* (2002) but relatively similar to Radcliffe *et al.* (1983) and Adeola *et al.* (1986). In comparison to wheat, McKenzie and Farrell (1980), Heger and Eggum (1991) and Flores *et al.* (1994b) have reported that triticale has a higher protein value. The crude fat and ash contents of the triticale cultivars in the current study were relatively more variable than those in other studies. With regard to the gross energy content, there was only a minor variation between the cultivars as well as between the years of harvest. The values are also similar to those obtained in South Africa and Canada (Adeola *et al.*, 1986; Brand *et al.*, 2003).

3.4.2 Starch composition and properties

In previous studies, starch contents of triticale varied from 630 to 730 g/kg DM (Leon *et al.*, 1996; Hughes and Cooper, 2002; Çiftci *et al.*, 2003). The contents found in the current study were lower than these values, the highest starch content being in cultivar H127, a trial name of triticale cultivar that was later commercialised as 'Bogong'. Although all the triticale samples from the two harvest years contained less total starch than was found in earlier studies, the lower content of resistant starch found in the triticale samples demonstrated that a large proportion of starch fraction may be digestible.

In the current study, the composition of amylose and amylopectin varied from 138 to 180 g/kg and from 442 to 500 g/kg, respectively. The highest amylose content was recovered in H249 cultivars. Moran (1982b) and Tester *et al.* (2004) defined a 'normal' amylose content as being between 160 and 350 g/kg, and the starch is regarded as 'waxy' or 'amylo-' when the amylose content is outside this range. Based on these definitions, the cultivars H249, AT528, H20, H127, H128 and H157 can be categorised as 'normal' while H418, JRCT74, H426, and Tahara cultivars, which contained less than 160 g/kg amylose are 'waxy' cultivars. Cultivar H418 is a trial name of a cultivar that is now commercialised as 'Canobolas'. In terms of amylose content, all the cultivars are potentially of high digestibility in view of their 'low' amylose contents. Oates (1997) and Carré (2004) postulated that starch with high amylose content (>40%) is often considered to be of lower digestibility, i.e. the starch becomes more resistant.

A sufficient number of representative granules were not measured to enable detailed statistical analysis, but there appeared to be little difference between the cultivars in starch granular structure. The shape of the starch granules was also similar to that of the parent grains, wheat and rye, which tend to show a bimodal distribution of granule sizes in the mature endosperm of the seed, corresponding with large A- and small B-type starch granules. However the further analysis was not conducted to determine amylose and amylopectin of the starch, it is assumed that the higher amylose content from the triticale starch may be due to the presence of large-size granules. Kim *et al*. (2012) reported that amylose content is is largely responsible for the swelling, solubility and gel-forming properties of starch.

3.4.3 Non-starch polysaccharides

Triticale is more similar to wheat than rye in terms of NSP content. The major fraction of cereal NSP is arabinoxylans (O'Brien, 1999; Dervilly-Pinel *et al.*, 2001). The NSP level has a negative

correlation with carbohydrate digestibility (Leeson and Summers, 2001). Austin *et al.* (1999) stated that water-insoluble NSP are indigestible by birds whereas soluble NSP are potentially digestible. However, Graham *et al.* (1993) reported that soluble NSP increase digesta viscosity, which can affect the growth and performance of birds. The new high-yielding triticale cultivars might have less problems of viscosity owing to their low percentage of water-soluble NSP as it found in the current study, the NSP found in the triticale cultivars was mostly insoluble. Data obtained in this study suggest that the concentration of soluble NSP was five times less than that of insoluble NSP. Cultivar AT528 and Tahara had the highest soluble NSP of the cultivars, whereas H128 and H20 contained the highest level of insoluble NSP. In comparison with NSP content of conventional ingredients used in poultry diets, such as maize and soybean meal, reported by Meng and Slominski (2005), four of triticale cultivars (H426, H418, H157 and H249) can be classified as low water-soluble NSP cultivars, whereas the other cultivars were slightly higher in NSP content than maize and soybean meal. Based on the total NSP found in the current study, the most common sugars were arabinose and xylose. Digestibility of triticale by birds therefore would likely be beneficial if they were supplemented with microbial enzymes targeting arabinans and xylans, which are the polymers of arabinose and xylose, respectively.

3.4.4 Amino acid content

The amino acid profiles of ingredients are important in formulating poultry diets because amino acids are relatively expensive (D'Mello, 2003) and a balanced amino acid (AA) profile in the final diet will have a protein-sparing effect (Brandt *et al.*, 2000). The concentrations of all AA found in the current study were higher than those reported by Sikka *et al.* (1978), Huet *et al.* (1988) and Mosse *et al.* (1988). In addition, the AA concentrations found in these triticale cultivars were also higher than those reported by NRC (1994).

In most cereal grains, lysine is the ‘first limiting’ amino acid (Brown, 1989). These results showed that the lysine content of triticale cultivars was higher than for wheat or rye. This finding is in agreement with the results reported by Stallknecht *et al.* (1996). Moreover, Heger and Eggum (1991) and Mosse *et al.* (1988) reported that lysine levels have not decreased with decreasing protein content and have been reported to be proportionally higher when the protein content of grain was low. Furthermore, the content of lysine in some triticale cultivars was higher in the current study than reported by Flores *et al.* (1994b) and van Barneveld (2002). In

the present study, the highest lysine concentration was in the H249 cultivar, while the lowest was in H128.

Elliot (1995) concluded that, after lysine; methionine, threonine, tryptophan, isoleucine and arginine are the next limiting AA in most poultry diets. Tryptophan was not quantified in this study but the concentrations of other potentially limiting AA were higher in cultivars H157, H127, H418 and H249 compared to the other cultivars.

3.4.5 Mineral contents

Among the major elements, K content was the highest in the triticale cultivars investigated, followed by P, Mg, S, Ca and Na. The concentration of Mn was the highest among the trace elements, followed by Fe and Zn. The similarity in mineral composition between the cultivars as well as between triticale harvested in different years confirms the consistency of the cultivars tested. The difference in P between 2008 and 2009 may be due to external factors during the growing period, such as water supply, soil type and fertiliser. However, there was low variability in P content between the cultivars. In comparison with previous studies, the range of individual mineral contents corresponded with values reported by Lorenz *et al.* (1974) and Sehgal *et al.* (1983). Variations in concentration of mineral elements can be attributed to the effects of soil, water supply, time of sowing, fertilisers, as well as varietal differences. The values in this study indicate that triticale contains relatively higher amounts of the major elements K, P and Na than wheat. The Ca content, on the other hand, is relatively similar to wheat. In the same way, the important minor elements such as Zn, Cu, Fe and Mn were found to be present in higher amounts in triticale than in wheat as also reported by Lorenz *et al.* (1974).

3.4.6 Phytate-P content

The concentrations of phytate-P of triticale cultivars harvested in 2008 and 2009 were relatively similar. The levels of phytate-P were lower than those reported by Singh and Sedeh (1979) in a study of 14 cultivars of triticale, but they were in the range reported by Singh and Reddy (1977). In comparison, Kim *et al.* (2002) reported that the proportion of phytate-P to total P in wheat ranged from 55 to 79% in two harvest years. Extra attention should be paid to H157 cultivar when it is included in diets due to its high phytate-P content.

3.4.7 *In vitro* nutrient digestibility and viscosity of digesta

In the present study, *in vitro* digestibility of DM, starch and CP as well as the digesta viscosity were not different between the cultivars, or between the harvest years. These findings are positive for the rapid selection and use of the cultivars in diet formulation. However, the digestibility of starch is relatively lower than that in the study reported by Flores *et al.* (1994b). The presence of anti-nutritive factors, particularly NSP, may be responsible for the low digestibility of starch and protein. The inclusion of exogenous enzymes such as phytase and/or carbohydrase could help to increase the digestibility of these nutrients. Viscosity during digestion was relatively similar between the cultivars and this was mainly the result of the similarity in content and types of the NSP.

3.5 CONCLUSION

It may be concluded from the present study that the chemical compositions of the ten cultivars of triticale, harvested in two different years, was generally similar. Phosphorus content was the most variable between the two harvest years. The cultivars were superior, in terms of concentration of key nutrients such as protein and amino acids, to wheat and rye, the cereals from which triticale was bred. The nutritive value of these grains was tested in trials reported in the following chapters.

CHAPTER 4 THE ENERGY UTILISATION OF BROILER CHICKENS ON TRITICALE-BASED DIETS

4.1 INTRODUCTION

Recently released high-yielding triticale cultivars are better in nutrition than the earlier triticale cultivars, which had a nutritional value similar to wheat. Based on the results in Chapter 3, triticale cultivars seem to have a better balance of essential nutrients such as protein and amino acids and the potential to replace wheat and maize in diets for poultry chickens.

Triticale will become an increasingly useful cereal grain in areas where it can be produced in reasonable amounts. The advantages of triticale include high yield, drought tolerance and disease resistance (Todorov, 1988). More work is, however, needed to evaluate its nutritive value. The concentrations of nutrients in some of the new high-yielding cultivars are also variable and need to be documented. In order to justify a recommendation as a replacement for other cereals, there is a need to assess the energy value of diets based on triticale. Energy supply is the key role of cereals in poultry diets (MacLeod, 2002) and it is common to have cereal grains as the predominant component of most practical diets (Ravindran and Blair, 1991).

The present study was conducted to investigate the energy utilisation of broiler chickens on diets containing some cultivars of triticale and the effect of these cultivars on productivity compared with maize- and wheat-based diets.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design and diets

Based on the results of laboratory work and *in vitro* assays of the triticale cultivars in Chapter 3 as well as their availability, five cultivars of triticale were identified for the *in vivo* trial reported in this Chapter. Two cultivars, Bogong and Canobolas, were tested in the preceding *in vitro* assays. These cultivars were obtained from Viterra Australia, which has the Plant Breeders Rights to market the seeds. Cultivars Jackie, Tobruk and Endeavour were purchased from a local seed retailer. Semi-purified diets were formulated to contain 70-75% of the cultivars Bogong, Canobolas, Jackie, Tobruk and Endeavour. Two similar control diets contained maize and wheat instead of triticale. All diets were pelleted and formulated to be isocaloric and isonitrogenous and were offered without any microbial enzyme

supplementation. The diets were formulated using a least-cost formulation software, Concept4-Ed, Educational Version 8.01.01 (Agri-data, 2008). The seven semi-purified dietary treatments with their ingredient and nutrient composition are presented in Table 4.1.

4.2.2 Bird management

A total of 336 day-old male Cobb-500 broiler chickens (initial weight, 37.15 ± 0.90 g) obtained from a commercial hatchery (Baiada Poultry Pty Ltd, Tamworth, NSW, Australia) were used in this trial and were randomly allotted to the seven dietary treatment groups (Table 4.1). There were six replicates of each treatment and eight chicks per replicate. The other 12 day-old chicks were used to obtain baseline data (see Section 4.2.4) for the comparative slaughter technique purposes.

The chickens were raised in four brooder decks, each deck with four tiers. Each tier contained four cages, each measuring 60 x 42 x 23 cm. In order to balance the distribution by location of cages, only the top three tiers were used. The cages were set up in two climate-controlled rooms (two brooder decks in each room). The diets were pelleted and provided to the birds along with water *ad libitum.* The temperature was maintained at 35ºC for the first two days and gradually decreased to approximately 24ºC by 22 d. On day 18, three birds from each cage, of approximately the same weight, were transferred to metabolism cages to enable a total collection of excreta for the last four days of the trial period. Prior to transfer, feed intake and body weight were recorded. The rearing conditions for birds moved to the metabolism cages were similar to those remaining in the brooder cages, the latter being kept for assessment of feed intake and body weight. A schedule of 18 h of light and 6 h of dark was maintained at both locations.

Table 4.1 Ingredient and nutrient composition (g/kg) of each dietary treatment

Ingredients	Bogong	Canobolas	Jackie	Tobruk	Endeavour	Maize	Wheat
Triticale	730.0	730.0	750.0	730.0	730.0	700.0	720.0
Soycomil P	201.0	193.0	179.	201.0	199.0	244.0	210.0
Sunflower oil	31.0	31.0	30.0	31.0	30.0	20.0	30.0
Dicalcium phosphate	7.0	8.0	6.0	5.0	4.0	9.0	5.0
Limestone	18.0	21.0	22.0	21.0	22.0	15.0	22.0
DL-Methionine	5.0	7.0	4.0	4.0	4.0	4.0	6.0
Common Salt	5.0	7.0	6.0	5.0	8.0	5.0	4.0
Premix[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl-70%	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Nutrient Composition							
ME (MJ/kg)	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Crude protein	220.0	220.0	220.0	220.0	220.0	220.0	220.0
Crude fat	45.0	44.0	44.0	47.0	43.0	41.0	44.0
Crude fibre	25.0	25.0	25.0	25.0	25.0	24.0	25.0
Lysine	11.7	11.5	11.1	11.4	11.9	12.3	11.3
Methionine	4.6	4.7.0	4.6	4.5	4.6	4.8	4.8
Tryptophan	3.6	3.5	3.6	3.6	3.6	2.6	3.1
Calcium	9.3	10.6	11.3	10.0	10.9	9.8	10.1
Available P	5.4	4.7	6.1	5.0	4.9	5.5	4.5
Sodium	2.0	2.9	3.5	2.1	3.3	2.0	1.6
Chlorine	2.1	2.3	2.2	2.2	2.1	3.0	2.9

[1]Supplied per kg of diet (mg): vitamin A (as all-trans retinol): 3.6; cholecalciferol: 0.09; vitamin E (as d-α-tocopherol): 44.7, vitamin K_3: 2.0; thiamine: 2.0; riboflavin: 6.0; pyridoxine hydrochloride: 5.0, vitamin B_{12}: 0.2, biotin: 0.1, niacin: 50.1, D-calcium pantothenate: 12.0, folic acid: 2.0, Mn: 80.0, Fe: 60.0, Cu: 8.0, I: 1.0, Co: 0.3, and Mo: 1.0.

4.2.3 Growth performance and AME measurement

The combined body weight (BW) and feed intake (FI) of birds in each cage were recorded on a weekly basis. Mortality was recorded as it occurred to allow for corrections to be made to the feed intake.

The excreta of the birds in the metabolism cages were collected daily between 19 and 22 d. The excreta were dried at 80ºC over 24 h, pooled by cage and ground. Subsamples of excreta and ground feed sample of each dietary treatment were stored for later determination of gross energy.

4.2.4 Analytical methods and nutrient retention analysis

On day 0 (first day), to provide baseline nutrient data, the twelve birds remaining after others were allocated to treatments were weighed and then slaughtered by cervical dislocation, without feeding. These birds were the initial slaughter group. The samples were stored in the freezer until the processing day after the trial was completed.

At day 22, after collecting the last day's excreta and weighing the birds, the birds were fasted for about 4 h before being slaughtered. Two birds were randomly picked from each cage and weighed and then killed by cervical dislocation. The whole birds, including feather, head, feed and all organs were put in a plastic bag and stored at -20ºC until processing. These birds constituted the final slaughter group.

The frozen chickens were moved to a cool room (4ºC) one day prior to processing, to thaw. On the processing day, the twelve birds (initial slaughter group) were chopped and blended at once in a heavy-duty blender. The same procedure was also carried out on the birds from the final slaughter group. Approximately 200 g of the finely ground carcasses were then freeze-dried. After freeze drying, samples were first ground in a coffee grinder and then by a mortar and pestle to produce finely ground homogenous samples that were analysed for gross energy, crude fat and nitrogen contents.

The gross energy of the diets, excreta and ground carcase samples was determined using a bomb calorimeter as described in Section 3.2.5. Crude fat was determined using the Soxhlet apparatus and chloroform as a solvent (see Section 3.2.3 for the procedure). The nitrogen content of all samples was determined using a Leco FP-2000 analyser, with EDTA as a calibration standard (see Section 3.2.2) and a multiplication factor of 6.25 was used to calculate CP from the total N.

Nutrient accretion and other variables were calculated using the following equations as described by Olukosi *et al.* (2008) with slight modification for AME, which was calculated from data on total excreta collections.

The AME of the diet and its utilisation were calculated using the following formula:

a. $\text{AME}\left(\frac{\text{kJ}}{\text{kg}}\right) = \frac{(\text{GE diet x g feed intake}) - (\text{GE excreta x g excreta})}{\text{g feed intake}}$

b. Initial GE of carcase (GE_0) (kJ) =

   Carcase GE (kJ/g) x body weight of bird day 0 (g)

c. Final GE content in carcase (GE_i) (kJ) =

   Carcase GE (kJ/g) x body weight of bird day 22 (g)

d. Net energy for production (NEp) (kJ) = (GE_i) - (GE_0)

e. HP (Heat Production) (kJ) = MEI-NEp;
   where MEI is ME intake, which was calculated from the following equation:

f. MEI (kJ) = ME (kJ/g) x feed intake (g);

g. Energy retained as fat (RE_f) (kJ) = Carcase fat (g) x 38.2 kJ/g;

h. Energy retained as protein (RE_p) (kJ) = Carcase CP content (g) x 23.6 kJ/g;
   The values 38.2 and 23.6 kJ/g are energy values per g of fat and protein, respectively (Larbier and Leclercq, 1992),

i. Efficiency of ME use for energy retention (K_{RE}) = NEp/MEI;

j. Efficiency of ME use for lipid retention (K_{REf}) = RE_f/MEI;

k. Efficiency of ME use for protein retention (K_{REP}) = RE_p/MEI

4.2.5 Animal ethics and statistical analyses

The experimental protocol was approved by the University of New England Animal Ethics Committee (Approval No: AEC09/099). The data were analysed using a one-way ANOVA in Minitab® Version 16 (Minitab, 2010), and using diets as the factor. The significance of difference between mean values was determined by Fisher's least significant difference (LSD) test.

4.3 RESULTS

4.3.1 Gross response

Over the first week on the experimental diets, FI did not differ (P >0.05) between groups; however, BW of the birds fed on the five diets containing triticale cultivars or maize were significantly higher (P <0.01) and FCR was lower (P <0.01) than for those given the wheat diet (Table 4.2). At 14 days of age, significant (P <0.01) differences were observed in all parameters measured between the dietary treatments. Birds fed triticale and maize diets ate more, were heavier and had a better FCR than those on the wheat diet. Among the triticale diets, birds given Bogong, Jackie, Tobruk and Endeavour cultivars had higher (P <0.05) BW than birds offered the Canobolas diets; however, all the birds fed triticale had similar FCR, which were better than the birds fed wheat. Feed intake to 21 days of age and BW at 21 d of the birds receiving Bogong, Jackie, Tobruk, Endeavour and maize were higher (P <0.05) than for birds fed the diets containing wheat and Canobolas cultivar, whereas there were no differences (P >0.05) in FCR between the treatments.

Table 4.2 Gross response of broiler chicken given diets based on triticale cultivars up to 7, 14 and 21 days of age[1]

Items	Bogong	Canobolas	Jackie	Tobruk	Endeavour	Maize	Wheat	SEM[2]
1-7 d								
FI (g/bird)	117.0	108.0	106.0	109.0	111.0	105.0	105.0	1.8
BW (g/bird)	123.0^{a}	115.0^{a}	117.0^{a}	118.0^{a}	117.0^{a}	123.0^{a}	101.0^{b}	1.6**
FCR (g/g)	1.38^{b}	1.40^{b}	1.38^{b}	1.36^{b}	1.37^{b}	1.22^{b}	1.66^{a}	0.029**
1-14 d								
FI (g/bird)	401.0^{a}	330.0^{d}	386.0^{b}	373.0^{ab}	362.0^{ab}	357.0^{bc}	313.0^{d}	5.7**
BW (g/bird)	338.0^{a}	284.0^{b}	327.0^{a}	316.0^{a}	320.0^{ab}	320.0^{a}	244.0^{c}	6.1**
FCR (g/g)	1.33^{b}	1.34^{b}	1.33^{b}	1.34^{b}	1.31^{b}	1.27^{b}	1.53^{a}	0.021*
1-21 d								
FI (g/bird)	777.0^{a}	571.0^{cd}	712.0^{a}	662.0^{bc}	696.0^{ab}	656.0^{bc}	503.0^{d}	1.87**
BW (g/bird)	690.0^{a}	547.0^{bc}	677.0^{a}	642.0^{a}	681.0^{a}	624.0^{ab}	462.0^{c}	1.67**
FCR (g/g)	1.19	1.13	1.11	1.11	1.08	1.12	1.19	0.016

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean.

[a-d]Values with unlike superscripts within a row are significantly different at *P <0.05 and **P <0.01.

4.3.2 Energy utilisation

The ME content of diets and intake of ME, gross energy, fat and protein by birds are presented in Table 4.3. The ME content differed between diets (P <0.01), from 11.4 MJ/kg for the diet containing Endeavour to 13.3 MJ/kg for the diet containing wheat. Between the diets containing triticale, the ME of the birds on Bogong and Tobruk-based diets were higher (P <0.01) than Canobolas, Jackie and Endeavour. In addition, the birds fed Canobolas and Jackie-based diets were higher (P <0.01) than Endeavour. The ME intake of birds on these diets, however, was not related to their ME content. The birds on the wheat-based diet, which had the highest ME content, had the lowest (P <0.01) ME intake, similar to that for the Canobolas diet, whereas the birds offered the diets with Bogong, Jackie and Tobruk had the highest (P <0.01) ME intake. These results were similar for gross energy, fat and protein intake, which were higher (P <0.01) on four of the five diets containing triticale and maize than on the diet containing wheat. Based on the result on Table 4.3, the birds on the Bogong-based diet performed better than all other triticale diets, however, birds fed Canobolas diets generally had a poorest performance.

Table 4.3 Metabolisable energy of diets (MJ/kg) and intake of ME (MJ), gross energy (MJ), fat (g) and protein (g) (per bird)[1]

Items	Bogong	Canobolas	Jackie	Tobruk	Endeavour	Maize	Wheat	SEM[2]
ME	13.0^{a}	12.3^{bc}	12.1^{c}	12.9^{a}	11.4^{d}	12.8^{ab}	13.3^{a}	0.12**
ME intake	13.6^{a}	9.5^{d}	12.1^{ab}	12.0^{ab}	10.2^{cd}	11.8^{bc}	9.4^{d}	0.31**
Energy Intake	17.9^{a}	12.9^{c}	16.8^{ab}	15.8^{b}	15.1^{b}	15.3^{b}	12.1^{c}	0.39**
Fat intake	47.1^{a}	34.7^{cd}	43.4^{ab}	43.6^{a}	38.3^{bc}	37.9^{c}	31.5^{d}	1.05**
Protein Intake	231.4^{a}	169.7^{cd}	219.0^{ab}	203.9^{b}	196.7^{bc}	202.7^{b}	156.0^{d}	5.04**

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean.

[a-d]Values with unlike superscripts within a row are significantly different at **P <0.01.

Figure 4.1 shows the influence of the experimental diets on the NEp and HP of the broiler chickens from hatch to 22 d.

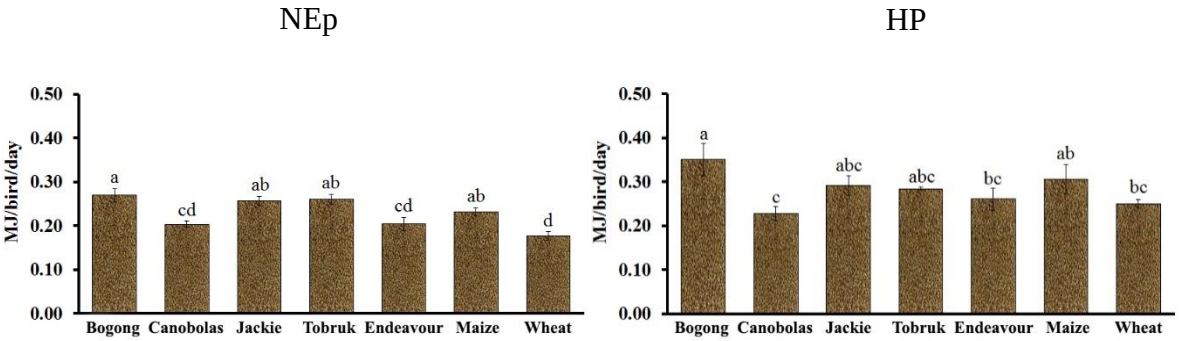

Figure 4.1 Net energy of production (NEp) and heat production (HP) (MJ/bird/day) of birds on diets based on different triticale cultivars between hatch and 22 d of age.

The NEp consumed by the birds fed on the diet containing Bogong, Jackie, Tobruk and maize was the same, and was higher (P <0.01) than NEp consumed by the birds on the Canobolas, Endeavour and wheat diets. In terms of heat production (HP), birds fed on the diets containing Jackie, Tobruk, Endeavour, maize and wheat had a similar production of the heat, meanwhile the birds on Bogong-based diet was similar to the birds on maize-based diet but higher (P <0.01) than all other treatments.

The whole body energy, fat and protein contents at 22 d of age are shown in Table 4.4. There were significant dietary effects on energy, fat and protein contents of the carcase. The energy content of the carcasses ranged from 3.9 to 5.9 MJ per bird on day 22. The energy content of carcasses of birds fed the Bogong diet was similar to that on the Jackie and Tobruk, but higher (P <0.01) than of all other treatments. A similar trend could be seen in the fat and protein contents of the carcase, which the fat content of the birds on the Bogong diet was similar to that on the Jackie and Tobruk. The protein content on the birds on Bogong, Jackie, Tobruk and maize were similar, but higher (P <0.01) than Canobolas, Endeavour and wheat diets.

Table 4.4 Whole body energy (MJ), fat (g) and protein (g) contents per bird at 22 days of age[1]

Items	Bogong	Canobolas	Jackie	Tobruk	Endeavour	Maize	Wheat	SEM[2]
Energy	5.9[a]	4.5[cd]	5.6[ab]	5.7[ab]	4.5[cd]	5.1[bc]	3.9[d]	0.14**
Fat	72.7[a]	52.0[bc]	68.7[a]	72.2[a]	47.2[bc]	57.7[b]	42.7[c]	2.19**
Protein	131.2[a]	104.0[cd]	124.7[ab]	121.9[ab]	111.8[bc]	120.3[ab]	93.5[d]	2.57**

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean.

[a-d]Values with unlike superscripts within a row are significantly different at $^{**}P < 0.01$.

Table 4.5 shows the influence of the different diets on the rate of deposition of energy, fat and protein from hatch up to day 22 of the experimental period. The daily rate of deposition of energy varied from 0.18 to 0.27 MJ/day, while fat and protein deposition ranged from 1.9 to 3.3 g/day and 4.3 to 6.0 g/day, respectively. These were significantly affected ($P < 0.001$) by diet.

Table 4.5 Rate of deposition of energy (MJ), fat (g) and protein (g) per day in broiler chicks grown to 22 days of age on diets based on triticale cultivars, maize or wheat[1]

Items	Bogong	Canobolas	Jackie	Tobruk	Endeavour	Maize	Wheat	SEM[2]
Energy	0.269[a]	0.203[cd]	0.256[ab]	0.260[ab]	0.203[cd]	0.232[bc]	0.177[d]	0.01**
Fat	3.3[a]	2.4[bc]	3.1[a]	3.3[a]	2.1[bc]	2.6[b]	1.9[c]	0.10**
Protein	6.0[a]	4.7[cd]	5.7[ab]	5.5[ab]	5.1[bc]	5.5[ab]	4.3[d]	0.12**

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean.

[a-d]Values with unlike superscripts within a row are significantly different at $^{**}P < 0.01$.

The birds on the Bogong, Jackie and Tobruk diet had higher ($P < 0.001$) rate of deposition of energy and fat than the other diets. Meanwhile, the rate deposition of protein, the birds on the Bogong, Jackie, Tobruk and maize diet had higher ($P < 0.001$) than the birds on Canobolas, Endeavour and wheat diets. In addition, the birds on the wheat diets had the lowest in all parameters measured (the rate of deposition of energy, fat and protein).

The results of energy retention as fat (RE_f) and protein (RE_p) are shown in Figure 4.2. Energy retention as fat ranged from 0.07 to 0.13 MJ/bird/day, and as protein from 0.10 to 0.14

MJ/bird/day. The energy retention as fat in the birds on the Jackie and Tobruk diets was similar to those on the Bogong diet, but higher (P <0.01) than for the Canobolas- and Endeavour- as well as the maize- and wheat-based diets. Similar to energy retention as fat, the birds in the Bogong, Jackie and Tobruk groups diet had higher (P <0.01) retention of energy as protein than other groups. The birds provided with the wheat diet had the lowest energy retention as fat and protein, but this was not statistically different from the Canobolas group.

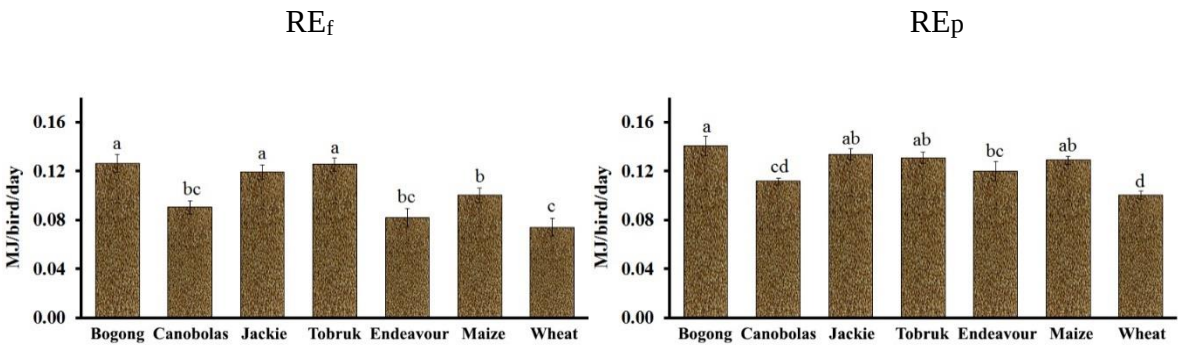

Figure 4.2 Energy retention as fat (RE_f) and protein (RE_p) (MJ/bird/day).

Figures 4.3 shows the efficiency of utilisation of ME (K_{RE}), fat (K_{REf}) and protein (K_{REp}), respectively. The K_{RE} and K_{REp} of the birds receiving different diets were not different (P >0.05), however, K_{REf} differed (P <0.05) between diets. The K_{RE}, K_{REf} and K_{REp} varied from 0.41 to 0.44, 0.17 to 0.22, and 0.23 to 0.26, respectively. The birds on the Tobruk diet were the most efficient (P <0.05) in using the ME for fat retention, whereas the birds receiving the diets based on Endeavour, maize, and wheat were less efficient in use of ME for fat retention.

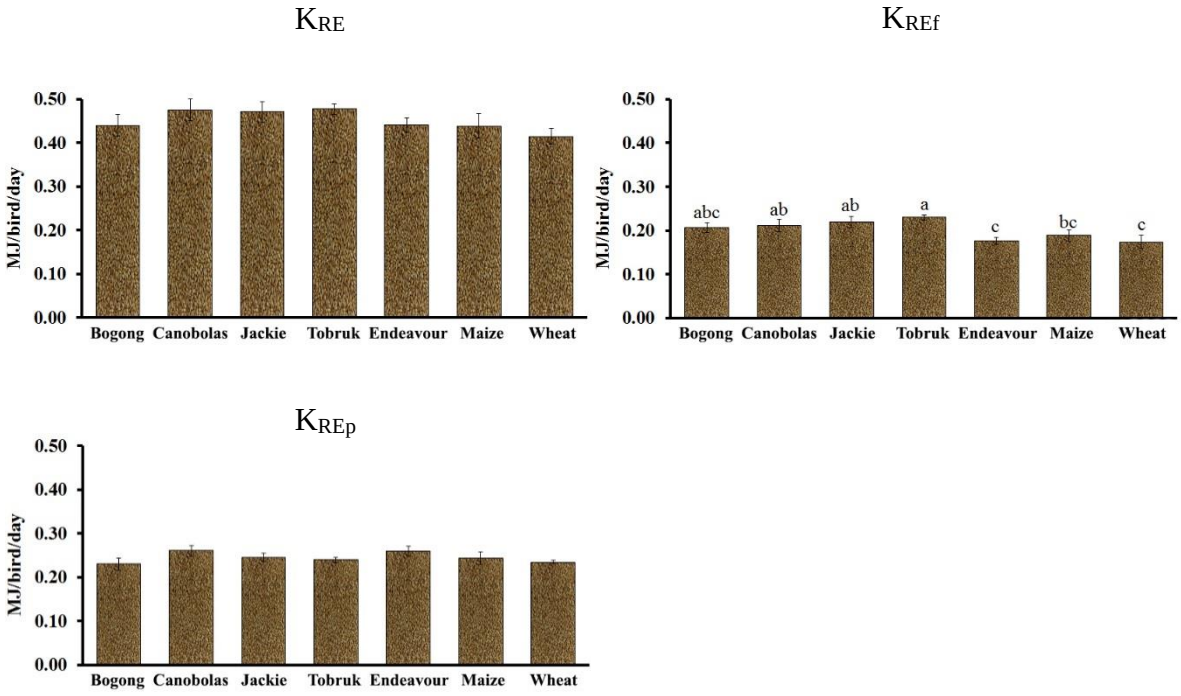

Figure 4.3 Efficiency of ME utilisation for energy (K_{RE}), fat (K_{REf}) and protein (K_{REp}).

4.4 DISCUSSION

In the present study, the gross responses expressed as FI, BW and FCR were affected by the dietary treatments. Up to day 21, all the birds were in good health. The mortality rate was less than 4% and mortality occurred only in the first week of the experimental period, possibly as birds adjusted to the diets and to other rearing conditions. The body weight was lower than the Cobb breeder specification (Teeter and Wiernusz, 2003), e.g. the highest BW to day 21 was about 8% less than the guide value. The lower value may be due to lower starting weight, which was about 15% less than the guide. The diets were also fed without microbial enzyme supplements and the lack of such supplementation was most obvious on the performance of birds on the wheat-based diet.

The feed intake of the birds up to day 7 did not differ between diets; however, feed intake from 0 to 14 and 0 to 21 days differed between diets. The fact that there was no difference in feed intake between groups up to day 7 may be the result of the birds' dependence on their residual yolk sac. This is in agreement with Turro *et al.* (1994) and Malik *et al.* (2011) who suggested that the residual yolk sac has the role of supplying some nutrients to the newly hatched chicken, especially in the first four days post-hatch. The demand for dietary nutrients would increase as the yolk became depleted, and dietary effects would become more obvious. The results in the

present study showed that the triticale cultivars contain more net energy and nutrients than wheat. Furthermore, by having the higher body weight, the diets containing triticale were possibly better digested than diets based on conventional grains, such as wheat. Barteczko *et al.* (2008) reported that nutrient and energy digestibility of grains depends on the chemical composition and higher nutrient content which can also lead to better digestibility.

Metabolisable energy is the standard measure of describing energy requirements for poultry and it is widely used in measuring energy contribution from poultry under commercial conditions (Lopez and Leeson, 2008). Metabolisable energy can be accurately determined from the difference between the gross energy of the feed and the gross energy of the excreta resulting from a feed (NRC, 1994). In the current study, except for the birds fed Endeavour, the ME content of the other triticale-, maize- and wheat-based diets was between 12 and 13 MJ/kg. ME intake was lower in birds fed Canobolas and wheat-based diets, compared with the other triticale cultivars and maize-based diets. The lower ME intake of the birds may be a result of the high fibre content and anti-nutritive factors present in Canobolas and wheat. Barteczko *et al.* (2009) reported that the soluble NSP fraction has a negative relationship with AME owing mainly to an increase in the digesta viscosity. Choct and Annison (1992), in a study on the anti-nutritive activity of isolated wheat pentosans, also reported a significant dose-dependent depression in AME. Other factors that possibly led to a reduction in the nutrient and energy utilisation by the birds is the presence of anaerobic microflora in the small intestine, which could affect the degradation of dietary components, corresponding to anti-nutritive effect of soluble NSP (Choct *et al.,* 1996) and the availability and digestibility of feed nutrients (Steenfeldt *et al.,* 1995; Smits *et al.,* 1997). Although the previous laboratory analysis of the triticale cultivars showed a similarity in nutrient contents between the triticale cultivars (results in Section 3.3), in this trial, the difference in ME, energy, fat and protein intake may be the result of the difference in digestibility between the triticale cultivars. However, predictions of digestibility from proximate composition may not be as accurate as values obtained from actual feeding trials.

The measurement of NEp has been used in this study to determine how useful the different grains were as energy sources. In the current study, NEp was significantly different between the treatments. The higher NEp in Bogong, Jackie and Tobruk diets compared to the NEp of the wheat diet, indicated a superiority of the three cultivars in terms of nutritive quality. However, the HP was substantially higher for all dietary treatments. These changes in HP are

similar to the findings of Olukosi *et al.* (2008) and Pirgozliev *et al.* (2011) who reported that HP was higher than NEp in all their experimental diets; however, the higher HP in their studies may be related to the inclusion of microbial enzymes in the diet (Olukosi *et al.*, 2008). Furthermore, Spratt *et al.* (1990) argued that there is a contribution of events at the tissue level to the total heat production. Vital organs such as liver, lungs and gastro-intestinal tract may consume up to 30% of the fasting HP and the total cost of maintenance may take up to 75% of total fasting HP. In the current study, it was found that energy deposition in protein was higher than energy deposition in carcase fat up to 22 days of age. According to Olukosi *et al.* (2008), this may be another reason for the high HP and can be explained by the fact that more ATP is required to deposit 1 g protein than to deposit 1 g fat.

The results showed that energy retention, in protein was higher than energy retention in fat in birds grown to 22 days of age. The carcase of birds fed diets with Bogong, Jackie and Tobruk showed a higher energy retention as fat and protein than the carcasses of birds fed on the maize and wheat diets. It would be expected that more lean tissue was being accumulated in the carcase of chickens that retained more net energy as protein. However, in general, the higher retention of protein than fat in broiler chickens up to 22 days of age is probably the result of the broiler chickens at this age are in the fast growing phase and have not reached the phase at which fat deposition would become significant (Sanz *et al.*, 2000; Bregendahl *et al.*, 2002). Emmans (1995) outlined that as body weight increases, the quantities of body fat and protein increase at different rates, and Leenstra (1986) added that fat deposits increased faster in later life.

In the present study, the efficiencies of retention of ME, lipid and protein were higher than in the study of Olukosi *et al.* (2008). This may be the result of the difference in ME intake. A higher efficiency of nutrient retention would be required to support a high energy intake. However, in the current study, the efficiencies of ME utilisation for energy and protein were unaffected by dietary treatment. In terms of efficiency of utilisation of ME for fat deposition, all the birds fed triticale diets had higher values, and were as efficient as those on maize but more than birds on wheat. The high level of fat deposition is related to a high ME intake. This is in agreement with Yuan *et al.* (2008), who reported that consumption of a high-energy diet by the bird will increase fat accumulation in the muscle. Similarly an increase in energy:protein ratio in the diet may stimulate a higher deposition of fat (Wiseman and Lewis, 1998). In view of the fact that the experimental diets were isocaloric and isonitrogenous, it can be said that the

higher fat deposition of the birds fed on Bogong, Jackie and Tobruk diets is the result of a higher digestibility of energy, and maybe the higher digestibility of protein too, but this was not assessed in the current study.

4.5 CONCLUSION

The present study showed that broiler chickens fed on some triticale cultivars (Bogong, Jackie and Tobruk) performed better than the birds on the other diets. Birds in Bogong, Jackie and Tobruk diet groups also had higher energy, fat and protein intake; net energy for production; energy, fat and protein retention, while the efficiency of utilisation of ME for energy and protein by the birds was unaffected. There is a need to test these findings under practical diets conditions, in which bird productivity would be at breeder standard. Such conditions would include supplementation with microbial enzymes.

CHAPTER 5 ENERGY UTILISATION AND PRODUCTIVITY OF BROILER CHICKENS ON DIETS CONTAINING TRITICALE AND SUPPLEMENTED WITH MICROBIAL ENZYMES

5.1 INTRODUCTION

The preceding study, reported in Chapter 4, indicated that the gross response and energy utilisation of broiler chickens fed a maize-based diet or some cultivars of high-yielding triticale were superior to those of chicken on a wheat-based diet without enzyme. In order to investigate the possibility of enhancing the nutritive value of these diets and productivity of the birds, microbial enzymes were used in the study reported in this Chapter.

The inclusion of NSP-degrading enzymes, with predominantly xylanase activity, in poultry diets has been routine for the past 20 years (Silversides and Bedford, 1999b), with the main aim being to increase the feeding value of the diets. Many reports have demonstrated the performance benefits of using enzymes, including xylanase to reduce the anti-nutritional effects of NSP and improve the availability of energy and nutritive value in wheat-based diets (Pettersson and Åman, 1989; Classen and Campbell, 1990; Dusel *et al.*, 1998; Zyla *et al.*, 1999; Selle *et al.*, 2003). Some researchers have reported that the application of xylanase in maize-based diets can improve nutrient utilisation and performance of broiler chickens (Wyatt *et al.*, 1997; Wyatt *et al.*, 1999; Zanella *et al.*, 1999; Beg *et al.*, 2001; Iji *et al.*, 2003); however, there are only few reports of xylanase being added to triticale-based diets (Pettersson and Åman, 1988; Pourreza *et al.*, 2007).

Nowadays, xylanase is sometimes used in combination with other enzymes, to optimize the utilisation of energy and nutrients of the diet (Beg *et al.*, 2001). There is much evidence that phytase also improves protein and energy utilisation (Selle *et al.*, 2000), and when xylanase and phytase are both included in the diet, xylanase will reduce digesta viscosity and release the trapped nutrients within the cell wall matrix as well as increasing the access of phytase to its substrate, to facilitate the absorption of nutrients. In addition, Ravindran *et al.* (1999b) reported that the simultaneous inclusion of xylanase and phytase was useful in improving the AME and digestibility of dietary protein.

Bogong and Canobolas are the two high-yielding triticale cultivars tested previously, and each supported very different levels of production. The growth performance and energy utilisation of chickens fed the Bogong-based diet were generally similar to those offered maize; on the other hand, Canobolas supported the lowest level of production among the five cultivars of triticale tested. Based on that information, it was hypothesised that when these cultivars of triticale partly substituted for maize in a maize-based diet, and xylanase and phytase were added, the nutrient quality of the Canobolas–based diet would be improved. Therefore, the objectives of this study were to determine the effects of adding microbial xylanase on its own, or combined with phytase to diets in which maize had been partially substituted with triticale, and to observe the effects of these two treatments on the growth performance and energy utilisation of broiler chickens as well as the visceral organ weight.

5.2 MATERIALS AND METHODS

5.2.1 Enzymes

The microbial enzymes used in this study were provided by AB Vista® (Marlborough, UK). The xylanase preparation (Econase® XT), which contains thermostable endo-1,4-beta-xylanase, produced by *Trichoderna reesei*, was added to supply 160,000 BXU of xylanase activity per kg diet or 0.1 g Econase® XT per kg diet. The microbial phytase (Quantum® 2500), which is a 6-phytase from *E. coli*, was added to supply 500 FTU per kg diet or 0.2 g Quantum® 2500 per kg diet.

5.2.2 Experimental birds and diets

This experiment used a total of 396 day-old male Cobb-500 chickens (initial weight, 40.3 ± 0.34 g) obtained from a commercial hatchery (Baiada Poultry Pty Ltd, Tamworth, NSW, Australia). The birds were used as follows: 12 birds were slaughtered on day 0 to provide baseline data, the remaining 384 birds were distributed to eight dietary treatments: Diet 1 contains 65% maize without enzyme (M); Diet 2 contains 65% maize with xylanase (MX); Diet 3 contains 30% Bogong inclusion in maize-based diet without xylanase (MB); Diet 4 contains 30% Bogong inclusion in maize-based diet with xylanase (MBX); Diet 5 contains 30% Bogong inclusion in maize-based diet with xylanase and phytase (MBXP); Diet 6 contains 30% Canobolas inclusion in maize-based diet without enzyme (MC); Diet 7 contains 30% Canobolas inclusion in maize-based diet with xylanase (MCX) and Diet 8 contains 30%

Canobolas inclusion in maize-based diet with xylanase and phytase (MCXP). The dietary experiments were formulated using Least Cost Formulation System, Concept4-Ed, Educational Version 8.01.01 (Agri-data, 2008). The ingredient and nutrient composition is presented in Table 5.1.

It had been planned to source the cultivars from the UNE breeding group, which were tested in Chapters 3. However, those cultivars were not available in the volume required, so the grains required were sourced from by Viterra Australia, a company involved in commercial distribution of these triticale cultivars.

5.2.3 Bird management, growth performance and sampling procedures

Body weight and FI of birds in each cage were recorded on a weekly basis. Mortality was recorded as it occurred to allow corrections to be made to the feed intake and feed conversion ratio (FCR). Feed and water were offered *ad libitum* in each cage.

The birds were raised in four brooder decks which were set up in a climate-controlled room. The layout of cages was similar to that described in Section 4.2.2. The temperature was maintained at 35ºC for the first two days and gradually decreased to approximately 24ºC over 21 d. On day 18, three layers of plastic sheets were placed underneath each cage to collect the excreta. A layer of plastic and the excreta thereon was collected in the morning on days 19, 20 and 21. About 50 g a daily subsample of excreta was well-mixed and stored in a cool room. On the last day (day 21), all subsamples were mixed and a composite sample (50 g) was taken and stored at -20ºC before freeze-drying. The freeze-dried samples were ground and used for gross energy determination.

Table 5.1 Ingredient and nutrient composition (g/kg) of each dietary treatment

Ingredients	M	MX	MB	MBX	MC	MCX	MBXP	MCXP
Maize	650.0	650.0	370.0	370.0	375.0	375.0	370.0	375.0
Bogong			300.0	300.0			300.0	
Canobolas					300.0	300.0		300.0
Soybean Meal	198.0	198.0	200.0	200.0	195.0	195.0	201.0	197.0
Hamlet Protein 300	104.0	104.0	82.0	82.0	81.0	81.0	82.0	81.0
L-Lysine HCl	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sunflower Oil	1.0	1.0	2.6	2.5	3.2	3.1	2.7	2.6
Limestone	16.0	16.0	16.4	16.4	16.8	16.8	16.5	16.3
Dicalcium phosphate	12.7	12.7	12	12	11.5	11.5	10.5	10.3
Common Salt	5.9	5.9	5.0	5.0	5.0	5.0	5.0	5.0
Choline Cl-70%	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Xylanase		0.1		0.1		0.1	0.1	0.1
Phytase							0.2	0.2
Premix[1]	2.6	2.6	2.5	2.5	2.5	2.5	2.5	2.5
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient Composition								
ME (MJ/kg)	12.8	12.8	12.6	12.6	12.6	12.6	12.6	12.6
Crude protein	210.0	210.0	210.0	210.0	210.0	210.0	210.0	210.0
Crude fat	33.6	33.6	29.7	29.8	29.6	29.7	29.6	29.5
Crude fibre	25.1	25.1	25.7	25.6	25.7	25.6	25.7	25.6
Lysine	12.2	12.2	12.3	12.1	12.3	12.1	12.3	12.1
Methionine	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
Tryptophan	2.5	2.5	2.4	2.3	2.4	2.3	2.4	2.3
Calcium	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Available P	5.0	5.0	5.0	5.0	5.0	5.0	4.8	4.8
Sodium	2.4	2.4	2.0	2.0	2.0	2.0	2.0	2.0
Chlorine	2.0	2.0	2.5	2.4	2.5	1.4	2.5	1.4

[1]Supplied per kg of diet (mg): vitamin A (as all-trans retinol): 3.6; cholecalciferol: 0.09; vitamin E (as d-α-tocopherol): 44.7, vitamin K_3: 2.0; thiamine: 2.0; riboflavin: 6.0; pyridoxine hydrochloride: 5.0, vitamin B_{12}: 0.2, biotin: 0.1, niacin: 50.1, D-calcium pantothenate: 12.0, folic acid: 2.0, Mn: 80.0, Fe: 60.0, Cu: 8.0, I: 1.0, Co: 0.3, and Mo: 1.0.

5.2.4 Analytical method and nutrient retention analysis

The comparative slaughter technique was employed to determine energy utilisation, including measurements of nutrient retention, NEp, HP and efficiencies of utilisation of protein, fat and ME for energy retention. The calculation to determine energy utilisation are as described in Section 4.2.4, while ME was calculated as described by Olukosi *et al.* (2008):

$$ME = GEi - [GEo\ x\ \left(\frac{Ti}{To}\right)],$$

where *GEi* is gross energy intake (MJ/kg) in feed; *GEo* is the gross energy output (MJ/kg) in excreta, *Ti* is the concentration of TiO_2 in the diets; and *To* is the concentration of TiO_2 in the excreta.

5.2.5 Titanium dioxide analysis

The TiO_2 content of treatment diets and excreta samples was measured according to Short *et al.* (1996). Around 0.2 and 0.1 g of freeze-dried samples of feed and excreta samples, respectively were weighed into porcelain crucibles and then ashed for 13 h at 580ºC. The ashed sample in each crucible was then dissolved in 5 mL of 7.4 M H_2SO_4 and left for about 10 min to cool. The samples were then gently boiled for about 30 min at 200ºC and then raised to 250ºC for another 30 min until completely dissolved. After cooling, the solution was quantitatively transferred into 50 mL volumetric flask through filter paper (Whatman 541, hardened ashless, 90 mm Ø Cat No. 1541 090, Whatman International Ltd. Maidstone, England) and rinsed with Milli-Q water. Ten mL of hydrogen peroxide (30% v/v) was added to each flask and the contents were diluted to 50 mL with Milli-Q water and gently mixed by repeated inversion to avoid bubbles. An orange colour developed with the intensity dependent on TiO_2 concentration. Aliquots of the solutions obtained and similarly prepared standard solutions were analysed using Varian Cary 50 Bio UV-Visible Spectrophotometer (©Varian Australia Pty Ltd) 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.

5.2.6 Visceral organ weight

One bird per cage was humanely slaughtered and dissected on days 7 and 21. The digestive tract from the proximal end of the proventriculus to ileo-caecal junction was removed and divided into proventriculus and gizzard, duodenum, jejunum and ileum. The contents of the proventriculus and gizzard were removed before weighing, but the sections of the small

intestine were weighed with the digesta. The other visceral organs also weighed on day 7 were pancreas, liver, spleen, bursa of Fabricius and yolk sac. On day 21, the same visceral organs were weighed, except for the yolk sac, which had been resorbed.

5.2.7 Animal ethics and statistical analysis

The experimental protocol complied with the standards for animal experiments, and approval for this study was granted by the University of New England Animal Ethics Committee (Approval No: AEC10/098). The effect of the grain and enzyme supplementation and their interactions were analysed using a general linear model (GLM) procedure of Minitab® Version 16 (Minitab, 2010). The experimental design was limited by availability of pens, so a 3-way interaction between grain, xylanase and phytase was not calculated. Some, but not all diets, contained both xylanase and phytase and the enzyme combination was treated as a single factor. The significance of the difference between means was determined by Fisher's least significant difference (LSD) test, for which the significant levels was set at $P \leq 0.05$.

5.3 RESULTS

5.3.1 Gross response

Table 5.2 shows the performance parameters from days 1 to 7 and 1 to 21. None of the parameters measured; FI, BW, or FCR, was affected by the inclusion of xylanase, and there was no significant interaction between grain and xylanase; however, with the inclusion of xylanase, these values where marginally increased. The FCR of birds offered the maize diet from 1-21 days, was similar to the Bogong-substituted groups, meanwhile it was better ($P < 0.05$) than that of birds on the Canobolas-substituted diets, regardless of xylanase supplementation.

Table 5.2 Feed intake (FI), body weight (BW) and feed conversion ratio (FCR) of birds on maize or containing Bogong and Canobolas with or without the inclusion of xylanase between hatch and 7 or 21 d of age[1]

Treatments		1-7 days			1-21 days		
Grain	Enzyme	FI (g/bird)	BW (g/bird)	FCR (g/g)	FI (g/bird)	BW (g/bird)	FCR (g/g)
Maize	-	144.3	170.5	1.108	1347.5	1023.5	1.370^{c}
Maize	Xylanase	148.1	170.0	1.146	1365.3	1029.4	1.383^{c}
Maize + Bogong	-	147.4	169.9	1.139	1370.6	1028.9	1.387^{bc}
Maize + Bogong	Xylanase	153.5	174.8	1.141	1390.9	1039.5	1.393^{abc}
Maize + Canobolas	-	152.8	168.1	1.235	1399.5	1014.3	1.437^{a}
Maize + Canobolas	Xylanase	159.5	169.4	1.199	1407.2	1022.4	1.435^{ab}
Pooled SEM[2]		1.87	1.18	0.014	7.58	7.19	0.008
Source of variation		Significance of treatment effect					
Grain		ns	ns	ns	ns	ns	**
Xylanase		ns	ns	ns	ns	ns	ns
Grain x Xylanase		ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean; ns = not significant.

[a-c]Values with unlike superscripts within each column are significantly different at $^{**}P < 0.01$.

Although the inclusion of xylanase did not affect body weight and FCR (Table 5.2), the combination of xylanase and phytase in the Bogong and Canobolas diets increased the body weight and FCR (Table 5.3). Feed intake to 7 d was not affected ($P > 0.05$) by the combined inclusion of xylanase and phytase, although the values were numerically higher when the enzymes were included in the Bogong- and Canobolas-substituted diets. Meanwhile, BW was significantly increased ($P < 0.01$) by the inclusion of xylanase and phytase. The FCR was also 7.5% better ($P < 0.05$) when enzymes were added to the Canobolas-substituted diet while there was no statistically significant improvement for the Bogong-substituted diet.

On day 21, FI, BW or FCR were not significantly ($P > 0.05$) affected by the combined inclusion of xylanase and phytase. Feed intake was numerically increased by about 5 and 1% on the diets substituted with Bogong and Canobolas, respectively, owing to the combined inclusion of xylanase and phytase. The FCR was relatively unaffected on the Bogong-substituted diet but improved by 4% on the Canobolas-substituted diet. There was no significant interaction

between the grain and the combination between xylanase and phytase on LW and BW from 1 to 21 days.

Table 5.3 Feed intake (FI), body weight (BW) and feed conversion ratio (FCR) of birds given maize containing Bogong or Canobolas diets with or without the inclusion of both xylanase and phytase between hatch and 7 or 21 d of age[1]

Treatments		1-7 days			1-21 days		
Grain	Enzyme	FI (g/bird)	BW (g/bird)	FCR (g/g)	FI (g/bird)	BW (g/bird)	FCR (g/g)
Maize + Bogong	-	147.4	169.9^{ab}	1.14^{a}	1370.6	1028.9	1.39
Maize + Bogong	Xylanase + Phytase	161.3	180.8^{a}	1.15^{ab}	1444.5	1073.9	1.40
Maize + Canobolas	-	152.8	168.1^{b}	1.20^{a}	1399.5	1014.3	1.44
Maize + Canobolas	Xylanase + Phytase	153.1	178.3^{a}	1.11^{b}	1385.9	1042.9	1.38
Pooled SEM[2]		1.50	1.66	0.011	12.50	12.50	0.008
Source of variation		Significance of treatment effect					
Grain		ns	ns	ns	ns	ns	ns
Xylanase and Phytase		ns	**	*	ns	ns	ns
Grain x Xylanase and Phytase		ns	ns	**	ns	ns	ns

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean; ns = not significant.

[a-d]Values with unlike superscripts within each column are significantly different at *P <0.05, **P <0.01.

5.3.2 Energy utilisation

Table 5.4 shows the ME value of diets, and intake of ME, GE, protein and fat by the birds offered the different diets. The grain type and inclusion of xylanase did not significantly affect the intake of these dietary components, except for fat intake, which was higher (P <0.05) on the maize diets than in the diets containing triticale. Although inclusion of xylanase did not statistically affect ME and the intake of ME, GE, fat and protein, the values were numerically higher on all diets with xylanase than diets without xylanase. There was no significant interaction between the main effects on ME and the intake of ME, GE, fat and protein.

Table 5.4 Metabolisable energy content of diets based on maize or containing Bogong or Canobolas with or without the inclusion of xylanase (MJ/kg), and mean daily intake per bird of ME (MJ), gross energy (MJ), fat (g) and protein (g) of these diets by birds between hatch to 21 d of age[1]

Treatments		ME	ME Intake	GE Intake	Fat Intake	Protein Intake
Grain	Enzyme					
Maize	-	12.7	0.8	1.0	2.2[a]	13.5
Maize	Xylanase	12.8	0.8	1.1	2.2[a]	13.6
Maize + Bogong	-	12.6	0.8	1.1	1.9[b]	13.7
Maize + Bogong	Xylanase	12.6	0.8	1.1	2.0[b]	13.9
Maize + Canobolas	-	12.5	0.8	1.1	2.0[b]	14.0
Maize + Canobolas	Xylanase	12.5	0.8	1.1	2.0[b]	14.1
Pooled SEM[2]		0.04	0.01	0.01	0.02	0.08
Source of variation		Significance of treatment effect				
Grain		ns	ns	ns	**	ns
Xylanase		ns	ns	ns	ns	ns
Grain x Xylanase		ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.

[2]SEM = Standard error of mean; ns = not significant.

[a-c]Values with unlike superscripts within each column are significantly different at $^{**}P < 0.01$.

The inclusion of combination of xylanase and phytase on the diets containing Bogong and Canobolas did not significantly affect ($P > 0.05$) ME and the intake of ME, GE, fat and protein (Table 5.5); however, combined enzyme inclusion tended to increase dietary ME and the intake of ME, GE, fat and protein on the Bogong-containing diet. On the other hand, in the case of Canobolas, the values were numerically less on the diet with enzymes than in the diet without enzymes. Similar to the only xylanase inclusion diets' effect of interaction, there was no significant interaction between the grain and the inclusion of xylanase and phytase simultaneously.

Table 5.5 Metabolisable energy content (MJ/kg) of diets based on maize containing Bogong or Canobolas with or without the inclusion of both xylanase and phytase, and mean daily intake per bird of ME (MJ), gross energy (MJ), fat (g) and protein (g) of these diets by birds between hatch to 21 d of age[1]

Treatments		ME	ME Intake	GE Intake	Fat Intake	Protein Intake
Grain	Enzyme					
Maize + Bogong	-	12.6	0.8	1.1	1.9	13.7
Maize + Bogong	Xylanase + Phytase	12.6	0.9	1.1	2.0	14.4
Maize + Canobolas	-	12.5	0.8	1.1	2.0	14.0
Maize + Canobolas	Xylanase + Phytase	12.4	0.8	1.1	2.0	13.8
Pooled SEM[2]		0.03	0.01	0.01	0.02	0.13
Source of variation		Significance of treatment effect				
Grain		ns	ns	ns	ns	ns
Xylanase and Phytase		ns	ns	ns	ns	ns
Grain x Xylanase and Phytase		ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.
[2]SEM = Standard error of mean; ns = not significant.

Figure 5.1 shows the effect of dietary treatments on NEp and HP. No significant differences were found for either NEp or HP, were not significantly affected by inclusion of xylanase or a combination of xylanase and phytase in the maize-based diet as well as in the Bogong and Canobolas-containing diets.

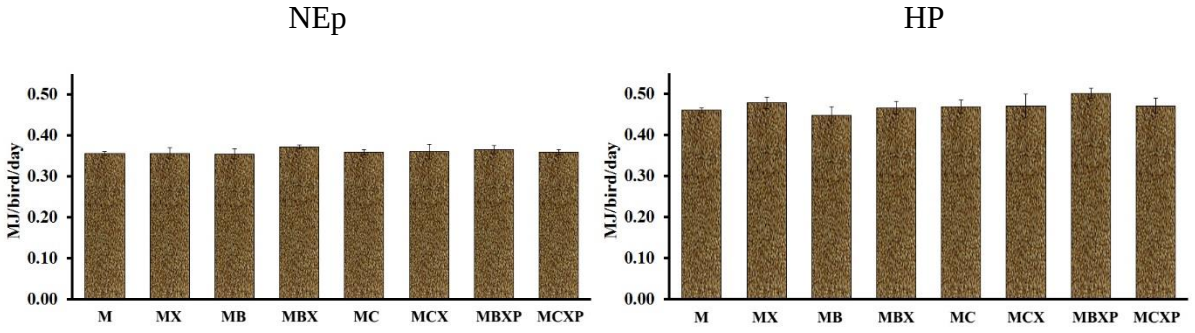

Figure 5.1 Net energy of production (NEp) and Heat Production (HP) (MJ/bird/day) of birds on different diets between hatch and 21 d of age.

The NEp value of maize diets (M and MX) and the Canobolas-containing diets (MC and MCX) had a similar value of 0.36 MJ/bird/day. The absolute value of NEp on the Bogong substituted

diet with xylanase was higher than without xylanase (0.37 vs. 0.35 MJ/bird/day). Meanwhile, the HP values in all diets were higher than NEp, which ranged from 0.45 to 0.50 MJ/bird/day.

The total energy, fat and protein contents of the whole body at 21 days and the mean daily rate of deposition of energy, fat and protein over 21 days are shown in Table 5.6. The values were not affected by dietary grain source or by the inclusion of xylanase. There was no significant interaction between the main effects on whole body energy, fat and protein retention.

Table 5.6 Whole body energy (MJ), fat and protein retention (g) per bird on different diets with or without xylanase until 21 d of age[1]

Treatments		Energy		Fat		Protein	
Grain	Enzyme	21 d	Daily	21 d	Daily	21 d	Daily
Maize	-	7.5	0.36	97.3	4.6	159.7	7.6
Maize	Xylanase	7.5	0.36	101.9	4.9	160.2	7.6
Maize + Bogong	-	7.4	0.35	97.7	4.7	166.2	8.0
Maize + Bogong	Xylanase	7.8	0.37	102.3	4.9	167.5	7.9
Maize + Canobolas	-	7.5	0.36	98.1	4.7	158.3	8.0
Maize + Canobolas	Xylanase	7.5	0.36	101.6	4.8	167.3	7.5
Pooled SEM[3]		0.09	0.004	1.24	0.06	1.98	0.09
Source of variation		Significance of treatment effect					
Grain		ns	ns	ns	ns	ns	ns
Xylanase		ns	ns	ns	ns	ns	ns
Grain x Xylanase		ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates; SEM = Standard error of mean; ns = not significant.

The fat and protein contents of the carcase from birds on all the diets with the inclusion of xylanase were higher than from diets without xylanase. The increase in fat content in the carcase of birds offered the maize-based diet, and the diets substituted with Bogong and Canobolas with xylanase were 4.47, 4.49 and 3.43% respectively, while for the protein, the increase was 0.79, 2.16 and 14.3%, respectively. The interaction between xylanase and phytase did not affect (P >0.05) the total energy, fat and protein contents of the whole body at 21.

Energy retention as fat (RE_f) and protein (RE_p) is presented in Figure 5.2. It shows that between hatch to 21 day, the mean daily of RE_f and RE_p of the broiler chickens were not statistically influenced by the dietary treatments.

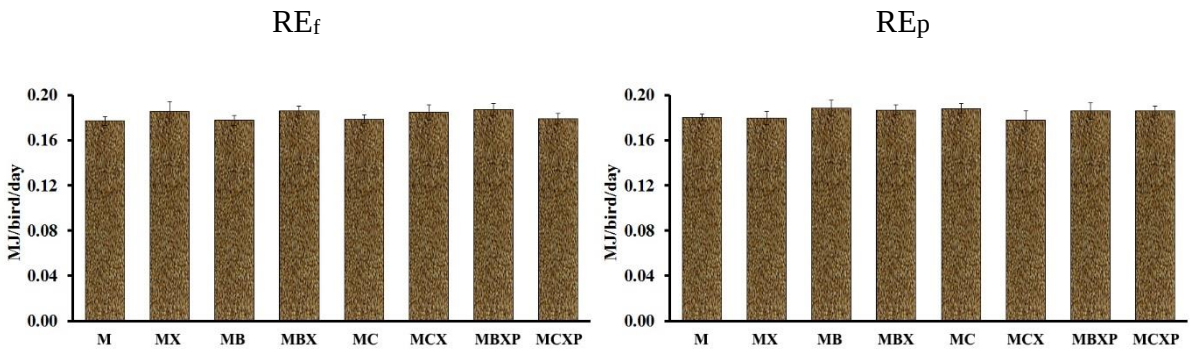

Figure 5.2 The energy retained as fat (RE_f) and protein (RE_p) (MJ/bird/day) in birds on different diets between hatch and 21 d of age.

The efficiency of utilisation of ME for energy (K_{RE}), fat (K_{REf}) and protein (K_{REp}) retention are shown in Figure 5.3. In the present study, K_{RE}, K_{REf} and K_{REp} were not affected by the type of grain, xylanase supplementation or the combination of xylanase and phytase. The values of K_{RE} in all dietary treatments were ranging from 0.421 (MBXP) to 0.445 (MBX), K_{REf} varied between 0.215 (MC) and 0.223 (MBX) and K_{REp} values were between 0.212 (MCX) and 0.230 (MB).

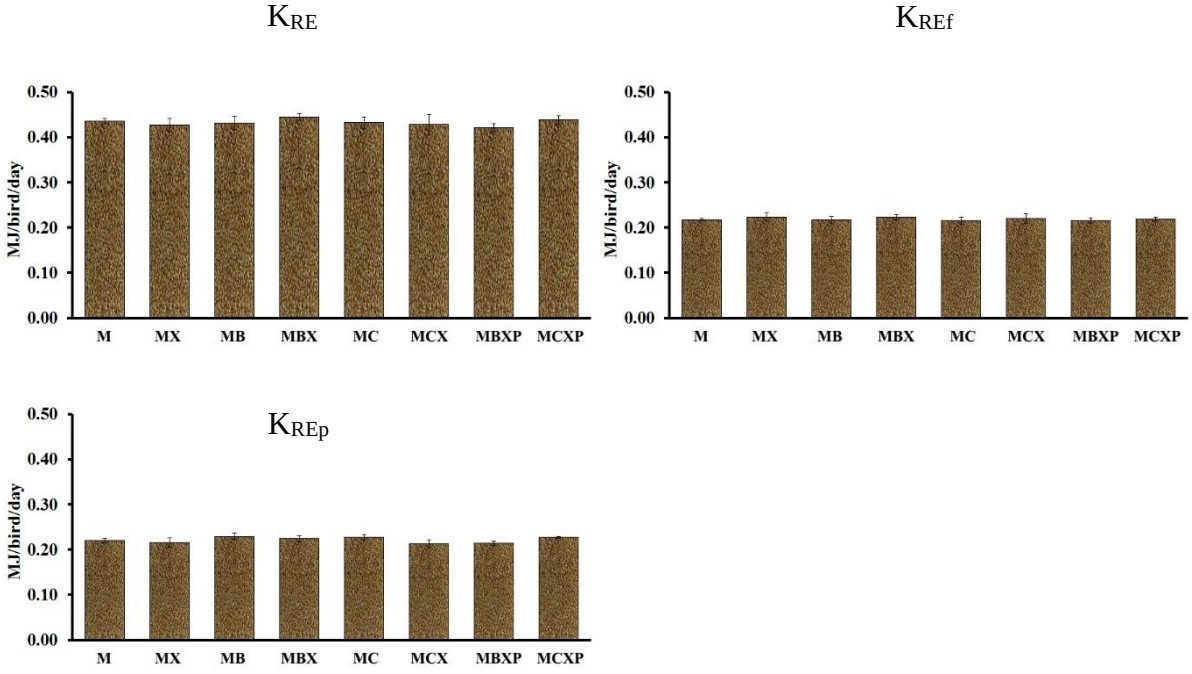

Figure 5.3 Efficiency of utilisation of ME for energy (K_{RE}), fat (K_{REf}) and protein (K_{REp}) retention by broilers on different dietary treatments between hatch and 21 d of age.

5.3.3 Visceral organ weight

The relative weights of the visceral organs of the chickens on days 7 and day 21 are presented in Tables 5.7 to 5.8. The weight of visceral organs on the two sampling days was not influenced (P >0.05) by the different grains or by xylanase inclusion in the diet; however, the relative weight of pancreas on day 7 tended to be higher (P =0.089) for birds on the maize diets than for the diets containing Bogong and Canobolas. There is a tendency of interaction (P =0.093) between grain and xylanase, which affected the weight of pancreas on day 7.

None of the organs weight on day 21 was affected by the inclusion of the combination of supplemental xylanase and phytase. The combined inclusion of xylanase and phytase in the diets containing Bogong and Canobolas did not significantly affect the relative weight of visceral organs on days 7 and 21. There was no significant interaction between the grain and the inclusion of combination xylanase and phytase on organs weight on day 21.

Table 5.7 Relative weight of visceral organs (g/100g of body weight) of broiler chickens on days 7/21 after being given diets based on maize or containing Bogong or Canobolas with or without xylanase[1]

Treatments		Proventriculus and gizzard	Small Intestine	Pancreas	Liver	Spleen	Bursa of Fabricius	Yolk sac
Grain	Enzyme							
Maize	-	5.7/3.6	5.7/3.3	0.48/0.29	4.7/3.3	0.07/0.08	0.13/0.18	0.08
Maize	Xylanase	6.3/3.5	5.8/3.3	0.49/0.29	4.8/3.3	0.07/0.08	0.14/0.18	0.07
Maize + Bogong	-	6.9/3.6	5.7/3.6	0.45/0.27	4.8/3.1	0.10/0.08	0.18/0.18	0.12
Maize + Bogong	Xylanase	6.3/3.5	5.5/3.7	0.48/0.26	4.7/3.3	0.10/0.09	0.14/0.17	0.06
Maize + Canobolas	-	5.3/4.1	5.9/3.8	0.46/0.27	5.1/3.2	0.06/0.09	0.14/0.15	0.14
Maize + Canobolas	Xylanase	6.5/3.7	5.5/3.8	0.39/0.29	5.6/3.2	0.07/0.10	0.15/0.17	0.04
Pooled SEM[2]		0.22/0.10	0.10/0.06	0.011/0.007	0.11/0.04	0.008/0.003	0.006	0.018
Source of variation				Significance of treatment effect				
Grain		ns/ns	ns/ns	0.089/ns	ns/ns	ns/ns	ns/ns	ns/ns
Xylanase		ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns
Grain x Xylanase		ns/ns	ns/ns	0.093/ns	ns/ns	ns/ns	ns/ns	ns/ns

[1]Each value represents the mean of 6 replicates.
[2]SEM = Standard error of mean; ns = not significant.

Table 5.8 Relative weights of visceral organs (g/100g of body weight) of broiler chickens on days 7/21 after being given diets based on maize containing Bogong or Canobolas with or without the combination of xylanase and phytase[1]

Treatments		Proventriculus and gizzard	Small Intestine	Pancreas	Liver	Spleen	Bursa of Fabricius	Yolk sac
Grain	Enzyme							
Maize + Bogong	-	6.9/3.6	5.7/3.6	0.45/0.27	4.8/3.1	0.10/0.08	0.18/0.18	0.12
Maize + Bogong	Xylanase + Phytase	6.4/3.8	5.6/3.5	0.45/0.25	4.9/3.1	0.08/0.08	0.17/0.17	0.11
Maize + Canobolas	-	5.3/4.1	5.9/3.8	0.46/0.27	5.1/3.2	0.06/0.09	0.14/0.15	0.14
Maize + Canobolas	Xylanase + Phytase	6.6/3.2	5.1/3.3	0.45/0.26	5.3/2.9	0.09/0.09	0.16/0.15	0.03
Pooled SEM		0.31/0.18	0.12/0.07	0.012/0.08	0.137/0.05	0.005/0.003	0.008/0.08	0.026
Source of variation				Significance of treatment effect				
Grain		ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns
Xylanase and Phytase		ns/ns	0.062/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns
Grain x Xylanase and Phytase		ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns

[1]Each value represents the mean of 6 replicates.
[2]SEM = Standard error of mean; ns = not significant.

5.4 DISCUSSION

5.4.1 Gross performance

The combined supplementation of xylanase and phytase to diets in which triticale was substituted for maize improved the nutritional value of the diets. There was a particularly marked improvement in the nutritional value of Bogong-based diets, which performed poorly without the inclusion of these enzymes, especially in the early stage of feeding.

The present study demonstrates that xylanase included on its own in the triticale-based diets did not improve feed intake or body weight, but when the diets were supplemented with a combination of xylanase and phytase, the performance of broiler chickens improved, in terms of body weight gain and feed efficiency; however, this only occurred in the young chicks up to day 7. It is likely that the phytase only influenced bird performance during this early stage of growth. This might be owing to the poor efficiency of nutrient utilisation by young birds resulting from the presence of a low level of endogenous phytase. In older birds, phytate hydrolysis could be improved through the activity of intestinal phytase, which is mainly of microbial origin. The population of such microbes is low in young chicks (Mitchell and Edwards, 1996). Phytate hydrolysis not only makes P more available but also other nutrients, including protein.

These performance results are slightly different from those of Ravindran *et al.* (1999b), Zyla *et al.* (1999) and Selle *et al.* (2003) who reported that the inclusion of a combination of xylanase and phytase to wheat- and barley-based diets improved the growth performance of adult broiler chickens, while in these trials, the growth performance was only slightly higher for diets containing both enzymes. The difference is probably due to the different grains used in previous and present studies. Nevertheless, there are some reports that suggest that the use of various exogenous enzyme preparations improves performance of birds offered maize-based diets (Wyatt *et al.*, 1997; Wyatt *et al.*, 1999; Zanella *et al.*, 1999; Beg *et al.*, 2001; Iji *et al.*, 2003). Maize contains a lower concentration of water-soluble NSP than temperate cereals (Choct, 1997).

5.4.2 Energy utilisation

In the present study, energy utilisation in general was not significantly influenced by xylanase on its own or the combination of xylanase and phytase; however, the energy values of the diets

with enzyme inclusion were slightly higher than those of the diets without enzymes. The results are slenderly different compared to the various previous studies (Zanella *et al.*, 1999; Gracia *et al.*, 2003; Cowieson and Adeola, 2005) that have demonstrated that the nutritive value of the grain can be improved by the inclusion of exogenous enzymes in the diet, which the inclusion of enzymes did not affect the performance indicators in this study. Fat intake was significantly higher on all maize diets than on the diets in which Bogong and Canobolas substituted for maize. This result is similar to that of Nian *et al.* (2011) who reported that the inclusion of xylanase in maize-soy-based diet did not affect diet AME. In contrast, Selle *et al.* (2003) found that AME content of a wheat-based diet was increased by the inclusion of a combination of supplemental xylanase and phytase. The mechanisms responsible for improved AME with the inclusion of phytase are unclear; however, the presence of xylanase in the diet may increase the AME, as xylanase can break down NSP in the diet and consequently improve the utilisation of energy and nutrients. The higher ME and fat intake of the control (all maize) diets than of the triticale substituted diets is related to the higher fat content of maize compared with the triticale. The crude fat content of maize is about 27.8 g/kg DM (NRC, 1994), whereas for triticale, it ranges from 13.9 to 20.0 g/kg DM (Widodo *et al.*, 2010). On the other hand, protein intake was higher on the triticale-containing diets and more energy was also retained in the form of protein than on the maize-based diets. This result might be due to better protein utilisation, which may increase the protein retention. Our findings for fat and protein intakes were in agreement with Barteczko *et al.* (2008) who reported that the ingredient concentrations in dietary components is related to the efficiency of nutrient digestion, which may occur on the maize control diet groups for crude protein and on the triticale diet groups for fat content, but not in the substituted diets.

Another measure of energy utilisation is NE, which is available to the animal to support both maintenance and production. This figure is the actual energy that is used by the body of the animal, after some part of the ME has been lost as heat. The measurement of NEp has been used by some workers to determine the efficiency of enzyme supplementation of the diet. Daskiran *et al.* (2004) found that carbohydrase supplements improved NE availability in maize-soybean meal diet, while ME intake was not changed. In the present study, NEp and HP were not affected by the inclusion of the microbial enzymes. This result is different with the findings of Olukosi *et al.* (2008), who reported that the improvements in energy deposition in the carcase may be due to increased nutrient and energy absorption.

The heat production of broiler chickens on the different dietary treatments did not differ significantly; however, HP on all the diets with the enzyme inclusion was numerically higher than that on the diets without added enzymes. These results are similar to the findings of Olukosi *et al.* (2008) who reported that HP was higher in broiler chickens given a diet with exogenous enzymes. The higher HP might result from the high accretion of protein in the present study, which was higher than fat deposition which contributes less HP than muscle (Close, 1990). In this connection, MacLeod (1991) similarly found that HP was higher in broiler chickens offered high-protein diet. Meanwhile, in the case of young piglets, Kies *et al.* (2005) reported that the higher HP on an enzyme-supplemented diet was the result of the maintenance of skeleton and fat, as well as energy associated with energy-dependent nutrient and mineral absorption processes. Consequently, the process may result in increased HP. Olukosi *et al.* (2008) also reported that with the use of phytase, some additional loss of energy as HP occurred owing to the increased release of nutrients and minerals which need to be actively transported through the gut and tissue cell walls.

The dietary treatments did not significantly affect energy, fat and protein deposition. The most obvious observation made was that protein retention (g) was higher than fat retention (g). This phenomenon is associated with the period of the treatment, namely the most rapid growth stage, which occurs before the stage at which fat deposition overtakes protein deposition (Sanz *et al.*, 2000; Bregendahl *et al.*, 2002). Wiseman and Lewis (1998) reported that an increase in calorie:protein ratio in the diets might stimulate higher abdominal fat accumulation in the older broiler chicken. A higher energy, fat and protein deposition was found in birds given the diets with supplemental exogenous enzymes. This could be caused by an increased nutrient digestibility as a result of more complete removal of anti-nutrients such as NSP and phytate, than was occurring with only the bird's endogenous enzymes. With regard to protein deposition and the role of xylanase in the diet, Choct and Kocher (2000) reported that carbohydrase disruption of the cell wall matrix or NSP linkages in the grain, improves access for the endogenous proteolytic enzymes that then assist the digestion of the previously entrapped proteins.

5.4.3 Relative visceral organs weight

The present study clearly indicates that the inclusion of xylanase in the diet had minimal effect on the relative organ weights. However, the influence of the combination between xylanase and phytase on the relative weight of the small intestine are not significantly different, there

was a higher relative weight of the organs in early life (up to 7 d) on the diet without xylanase and phytase supplementation. This finding is probably the result of phytase inclusion which has a greater effect in younger birds than older birds (Kornegay *et al.*, 1996).

5.5 CONCLUSION

The present study showed that the inclusion of xylanase on its own did not affect gross response, energy utilisation or relative organ weights of broiler chickens offered diets in which maize was substituted with two triticale cultivars, Bogong and Canobolas. There was an improvement in some measures of performance when phytase was included along with xylanase in the diets. There were no negative consequences when Bogong and Canobolas partially replaced maize in a maize-based diet; however, the birds tended to have better performance when microbial xylanase and phytase were added to these diets. These results support the suggestion that triticale (cultivars Bogong and Canobolas) has considerable potential as an alternative grain source for inclusion in diets for broiler chickens.

CHAPTER 6 RESPONSE OF BROILER CHICKENS TO DIETS BASED ON TRITICALE AND SUPPLEMENTED WITH MICROBIAL ENZYMES.
1. GROWTH AND INTESTINAL FUNCTION

6.1 INTRODUCTION

The results of the previous study (Chapter 4) showed that the birds given diets in which triticale wholly replaced maize and wheat, and without enzyme supplementation, performed better than birds on wheat-based diets and as well as birds on a maize-based diet. Currently, most practical broiler chicken diets contain microbial enzymes and their effects were tested and reported in Chapter 5, in which triticale partly substituted for maize in broiler chicken diets, and supplemented with microbial enzymes. Triticale's potential to replace maize (Zarghi and Golian, 2009) and wheat (Osek *et al.*, 2010), has been established. It would be important to test the response of poultry to diets solely based on the cultivars being tested in this project, when the diets are supplemented with microbial enzymes.

There have been some previous tests in which older cultivars of triticale constituted the only cereal grain in the poultry diet. The response to such diets was poor (Bragg and Sharby, 1970; Shimada *et al.*, 1974), and similar results have been observed for later cultivars of triticale (Salmon, 1984; Rundgren, 1988; Smith *et al.*, 1989). The low performance of the birds on such diets was attributed to the presence of NSP, which are mainly of arabinoxylans and β-glucans (Classen and Bedford, 1991; O'Brien, 1999; Dervilly-Pinel *et al.*, 2001; Pourreza *et al.*, 2007) and also phytic acid (Jondreville *et al.*, 2007). Annison and Choct (1991) and Bedford (1995) have suggested that supplementation with exogenous carbohydrase enzymes, such as xylanase, can reduce the viscosity of the intestinal contents and improve the digestibility of starch, protein and energy in broiler diets. Likewise, the inclusion of phytase in the broiler diet can increase feed utilisation and body weight, lower mortality and lower P content in faeces (Huff *et al.*, 1998; Levic *et al.*, 2006).

There has been a particular focus on dealing with the negative effects of its phytic acid content as well as the presence of xylans and arabinoxylans (Çiftci *et al.*, 2003; Jondreville *et al.*, 2007; Pourreza *et al.*, 2007; Zarghi and Golian, 2009). These studies show that the inclusion of enzyme preparations in the diet can improve chicken performance. In the study reported in Chapter 5, the triticale cultivars Bogong and Canobolas were used in place of maize in diets

for broiler chickens, and when supplemented with a mixture of xylanase and phytase led to improvement in bodyweight and energy utilisation. Moreover, Vieira *et al*. (1995) reported that the inclusion of up to 40% triticale in a maize-soy diet did not have any negative effect on body weight of broiler chickens. In addition, Fayez *et al*. (1996) reported that even when the diet contained 100% of a Syrian cultivar of triticale for the grain portion without inclusion of any enzymes, the productivity of broiler chicken did not decline. However, there is still no information about the physiological response of broiler chickens fed the newer high-yielding cultivars of triticale when supplemented with microbial enzymes.

The objective of this study was to examine the influence of supplementation with xylanase and phytase, individually or in combination, in diets based on these new cultivars of triticale (Bogong and Canobolas) on the gross response, visceral organ weight as well as some physiological responses.

6.2 MATERIALS AND METHODS

6.2.1 Enzymes

The microbial enzymes used in this study were supplied by AB Vista® (Marlborough, UK). The xylanase preparation, Econase® XT, which contains thermostable endo-1,4-beta-xylanase, produced by *Trichoderma reesei*, was added to supply 160,000 BXU of xylanase activity. The microbial phytase, Quantum® 2500, which is a 6-phytase from *E. coli* was added to supply 500 FTU per kg diet.

6.2.2 Experimental design and bird management

A 2 x 2 x 2 factorial arrangement was used to study 2 cultivars of high-yielding triticale (Bogong and Canobolas), with or without xylanase and with or without phytase. Each diet was formulated to contain triticale (650 g/kg) as the sole cereal grain. The dietary treatments were as follows: a diet based on Bogong without any enzymes (B); Bogong with the inclusion of xylanase (BX); Bogong with the inclusion of phytase (BP); Bogong with the inclusion of xylanase and phytase (BXP); Canobolas without enzymes (C); Canobolas with the inclusion of xylanase (CX); Canobolas with the inclusion of phytase (CP) and Canobolas with the inclusion of xylanase and phytase (CXP). The diets were pelleted and the ingredient and calculated nutrient composition of each diet is shown in Table 6.1.

A total of 384 day-old male Ross 308 broiler chicks (Baiada Poultry Pty. Ltd, Tamworth, NSW, Australia), weighing 41.30 ± 0.35 g, were randomly allocated to 48 cages. The experimental chickens were raised in battery brooders, 60 x 42 x 23 cm, set up in a climate-controlled room. Each of the 8 treatments was randomly assigned to 6 cages with 8 birds per cage. Water and feed were available *ad libitum.* The birds were initially brooded at a temperature of 34ºC, and this was gradually reduced to 24 ± 1ºC at 21 days of age when the feeding trial ended. Light was provided for 18 h per day throughout the experiment period.

The experimental diets (Table 6.1) were formulated to meet the minimum Aviagen Breeder recommendations (Aviagen, 2007). The experimental diets were comprising 65% of either Bogong or Canobolas. The rate of enzyme supplementation (in those diets that included enzymes) was 0.1 g Econase® XT per kg/diet and 0.2 g Quantum® 2500 per kg diet. An indigestible marker, TiO_2, was incorporated in all diets to enable nutrient digestibility of the diets to be determined.

On days 7 and 21, one bird and three birds, respectively, from each cage, were randomly selected, weighed and killed by cervical dislocation. The abdominal cavity was opened and the small intestine was ligated and removed. The purpose of sampling was to weigh the visceral organs and obtain a section of jejunum (approximately 5 cm of anterior part of jejunum, immediately distal to the posterior end of the duodenal loop) and pancreas for analysis of enzyme activities. For the determination of the TiO_2 content as well as nutrient digestibility, the digesta from the ileum were collected on day 21 sampling and pooled on a cage basis, homogenised and stored at –20ºC. Later, the samples were freeze-dried, ground with a small coffee grinding machine and stored in airtight containers at 4ºC until they were analysed to determine TiO_2, gross energy, starch and protein concentrations.

Table 6.1 Ingredient and nutrient composition (g/kg) of dietary treatments

Ingredients	B	BX	BP	BXP	C	CX	CP	CXP
Bogong	650.0	650.0	650.0	650.0				
Canobolas					650.0	650.0	650.0	650.0
Soybean Meal	190.0	190.0	190.0	190.0	190.0	190.0	190.0	190.0
Soycomil K	69.4	69.4	69.4	69.4	61.3	61.2	61.2	61.2
L-Threonine	1.8	1.8	1.8	1.8	1.9	1.9	1.9	1.9
L-Lysine HCl	4.8	4.8	4.8	4.8	5.3	5.3	5.3	5.3
DL-Methionine	2.6	2.6	2.6	2.6	3.0	3.0	3.0	3.0
Sunflower oil	35.7	35.7	35.5	35.3	42.6	42.6	42.5	42.4
Limestone	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1
Dical. P	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8
Common Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline Cl-70%	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5
Xylanase		0.1		0.1		0.1		0.1
Phytase			0.2	0.2			0.2	0.2
Premix[1]	2.6	2.6	2.6	2.6	2.5	2.5	2.5	2.5
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient composition								
ME (MJ/kg)	12.7	12.7	12.7	12.7	12.9	12.9	12.9	12.9
Crude protein	220.0	220.0	220.0	220.0	220.0	220.0	220.0	220.0
Crude fat	53.8	53.7	53.6	53.5	59.4	59.3	59.2	59.1
Crude fibre	25.5	25.5	25.5	25.5	25.2	25.2	25.2	25.2
Lysine	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Methionine	6.1	6.1	6.1	6.1	6.5	6.5	6.5	6.5
Tryptophan	2.9	2.9	2.9	2.9	2.8	2.8	2.8	2.8
Calcium	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Available P	5.2	5.2	5.2	5.2	5.6	5.6	5.6	5.6
Sodium	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chlorine	5.6	5.6	5.6	5.6	3.4	3.4	3.4	3.4

[1]Supplied per kg of diet (mg): vitamin A (as all-trans retinol): 3.6; cholecalciferol: 0.09; vitamin E (as d-α-tocopherol): 44.7, vitamin K_3: 2.0; thiamine: 2.0; riboflavin: 6.0; pyridoxine hydrochloride: 5.0, vitamin B_{12}: 0.2, biotin: 0.1, niacin: 50.1, D-calcium pantothenate: 12.0, folic acid: 2.0, Mn: 80.0, Fe: 60.0, Cu: 8.0, I: 1.0, Co: 0.3, and Mo: 1.0.

6.2.3 Measurements and analyses

Gross response

Feed intake (FI), BW, FCR and mortality were evaluated as described in Section 4.2.3. The viscosity of the ileal digesta was measured as described in Section 3.2.13.

Phytate-P content of ileal digesta

The phytate-P content of the diet and ileal digesta was collected quantitatively and measured as described in Section 3.2.12. In addition, the degradation of phytate (%), i.e. the percentage of dietary phytate-P apparently absorbed from the gut proximal to the ileum, was calculated by the following equation:

$$Phytate\ P\ degradation\ = \frac{(Phytate\ P_{(Diet)} - Phytate\ P_{(Digesta)})}{Phytate\ P_{(Diet)}}\ x\ 100$$

Visceral organ weight

On days 7 and 21, the visceral organs (small intestine, proventriculus plus gizzard with contents, liver, pancreas, spleen, and bursa of Fabricius) of the selected birds were obtained and weighed as described in Section 5.2.6. The body weight of the birds was recorded.

Tissue protein content and digestive enzyme analysis

The pancreas and the anterior part of the jejunum were placed on crushed ice within one minute of death. The jejunal tissue was then opened longitudinally along one side of the section, using a pair of sharp scissors and the mucosal surface was cleaned with 1% (w/v) physiological saline. The jejunal tissue and pancreas samples were then wrapped in a small sheet of labelled aluminium foil, and snap-frozen in liquid nitrogen. Samples were then stored at -20ºC until preparation for analysis.

The assessment of the digestive enzyme activities and protein concentration of the jejunal tissue was conducted as described by Shirazi-Beechey *et al.* (1991). The frozen tissue was weighed and cut into small pieces and defrosted in 20 mL buffer (100 mM mannitol, 2 mM HEPES/Tris, pH 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 homogenised using an IKA® Labortechnik homogeniser (Ultra Turrax T 25 Basic Homogeniser; IKA® Works,

Wilmington, NC, USA) at medium speed (2000 × **g**) for 30 seconds. Sub-samples of the homogenate were pipetted into two or three 1.5 mL microcentrifuge tubes (Eppendorf South Pacific, North Ryde, Australia) for storage at -20ºC, until enzyme analyses were conducted.

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 homogenised (Nitsan *et al.*, 1974). The homogenised tissue was then centrifuged at high speed (30,000 × **g**) for 20 min to obtain a crude homogenate supernatant.

The specific activities of jejunal and pancreatic enzymes were evaluated by incubation with fixed substrate concentrations as standardised for poultry by Iji *et al.* (2001). Assays on the jejunal homogenate 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); whereas assays on the pancreas were conducted for protein and chymotrypsin amidase (EC 3.4.21.1). The specific activities of enzymes were measured according to the methods described for other species (Holdsworth, 1970; Serviere-Zaragoza *et al.*, 1997) after standardisation for poultry. The measurement of alkaline phosphatase activity was conducted according to Forstner *et al.* (1968). The protein content of both jejunal mucosa and pancreatic tissue was measured according to Bradford (1976), using the Coomassie dye-binding procedure. The protein absorbance data obtained by colorimetry (using Varian Cary 50 Bio UV-Visible Spectrophotometer) were converted into absolute values using Lowry Software (Mepherson, 1985).

Ileal digestibility of nutrients

The TiO_2 contents of the ileal digesta and diet samples were measured by the method reported by Short *et al.* (1996), as described in Section 5.2.5. The TiO_2 marker concentrations in the feed and ileal digesta were used to calculate the digestibility coefficients for protein, gross energy, starch and minerals. Diets and ileal digesta were analysed for gross energy and starch, as described in Sections 3.2.5 and 3.2.6, respectively. The apparent digestibility coefficient (ADC) of nutrients was calculated using the following equation:

$$ADC\% = 100\, x \left(\frac{\%\, TiO2\ in\ feed}{\%\, TiO2\ in\ ileal\ digesta}\ x\ \frac{\%\, nutrient\ in\ ileal\ digesta}{\%\, nutrient\ in\ feed} \right)$$

Samples of diet and digesta were sent to Waite Analytical Services, University of Adelaide for mineral and nitrogen analyses.

6.2.4 Animal ethics and statistical analyses

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No. AEC 10/098). All data were analysed by ANOVA using the general linear model (GLM) procedure of Minitab® Version 16 (Minitab, 2010) for the main factors (cultivar, xylanase and phytase) and the interactions between these three factors. The significance of difference between means was determined by Fisher's least significant difference (LSD) test, for which the significant level was set at $P \leq 0.05$.

6.3 RESULTS

6.3.1 Gross response

The gross response of the birds fed Bogong- and Canobolas-based diets with and without xylanase and phytase is shown in Table 6.2. The feed intake to day 7 was increased ($P < 0.01$) by the inclusion of phytase to both diets. Feed intake to 21d was also slightly ($P = 0.063$) affected by the xylanase inclusion. Similar effects could be seen in the body weight of birds up to days 7 and 21. It was increased ($P < 0.01$) by the inclusion of phytase, in addition to the interaction ($P = 0.081$) between xylanase and phytase. The FCR to days 7 and 21 was not significantly affected by the treatments, but the FCR of birds on Bogongs-based was slightly ($P = 0.056$) better than on Canobolas-based diets. In addition, the best FCR was found in chicks on the diet containing only xylanase for both Bogong and Canobolas (1.04), which is to some degree better than the FCR on the Canobolas diet without enzyme and with both xylanase and phytase; which was 1.08 (or 3.9 % different).

There was no significant interaction between grain and xylanase for all parameters measured, except for a significant interaction ($P < 0.05$) between grain and phytase as well as between xylanase and phytase ($P < 0.01$) on the feed intake on day 21.

Table 6.2 Feed intake (FI), body weight (BW) and feed conversion ratio (FCR) of chickens on triticale-based diet with or without enzymes between hatch and 7 or 21 d of age[1]

Treatments			1-7 days			1-21 days		
Grain	Xyl[2]	Phy[3]	FI (g/bird)	BW (g/bird)	FCR (g/g)	FI (g/bird)	BW (g/bird)	FCR (g/g)
Bogong	-	-	146.3^{c}	180.8^{cd}	1.05	1008.7^{de}	813.7^{b}	1.31
Bogong	+	-	147.2^{c}	182.8^{cd}	1.04	1043.0^{d}	826.6^{b}	1.33
Bogong	-	+	167.5^{a}	201.7^{a}	1.04	1385.5^{a}	1071.9^{a}	1.35
Bogong	+	+	164.6^{ab}	198.3^{ab}	1.05	1275.6^{c}	1045.4^{a}	1.27
Canobolas	-	-	147.4^{c}	178.1^{d}	1.08	954.9^{e}	775.2^{b}	1.31
Canobolas	+	-	154.1^{bc}	189.1^{bc}	1.04	961.1^{e}	788.0^{b}	1.29
Canobolas	-	+	168.3^{a}	201.2^{a}	1.05	1373.6^{ab}	1066.0^{a}	1.34
Canobolas	+	+	170.8^{a}	199.4^{a}	1.08	1305.1^{bc}	1048.4^{a}	1.30
Pooled SEM[4]			1.94	1.79	0.005	27.00	20.20	0.010
Source of variation			Significance of treatment effect					
Grain			ns	ns	0.056	ns	ns	ns
Xylanase			ns	ns	ns	0.063	ns	ns
Phytase			**	**	ns	**	**	ns
Grain x Xylanase			ns	ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	*	ns	ns
Xylanase x Phytase			ns	0.081	0.067	**	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-d]Values with unlike superscripts within each column are significantly different at *P <0.05; **P <0.01.
ns = not significant.

6.3.2 Nutrient digestibility

The ileal digestibility of CP did not differ (P >0.05) between the cultivars, but was increased by the inclusion of xylanase and the interaction between the grain and xylanase (P <0.05), the inclusion of phytase, and the interaction between xylanase and phytase (P <0.01). The digestibility of CP increased by 9.4% with the inclusion of phytase in the Bogong diet, while

the inclusion of the combination of supplemental xylanase and phytase increased by 11.5% in the Canobolas diet (Table 6.3).

Table 6.3 The ileal digestibility (%) of CP, gross energy, starch, Ca and P of chickens on triticale-based diets with or without enzymes at 21 days of age[1]

Treatments			Crude protein	Gross energy	Starch	Ca	P
Grain	Xyl[2]	Phy[3]					
Bogong	-	-	77.7^{c}	78.9^{d}	83.5^{c}	41.3^{b}	43.8^{bc}
Bogong	+	-	81.0^{b}	81.6^{c}	86.3^{ab}	46.4^{ab}	49.6^{ab}
Bogong	-	+	85.0^{a}	84.0^{ab}	86.8^{ab}	45.8^{ab}	56.4^{a}
Bogong	+	+	82.2^{ab}	82.9^{abc}	85.6^{b}	44.1^{ab}	55.0^{a}
Canobolas	-	-	75.8^{c}	77.0^{e}	83.0^{c}	32.2^{c}	39.4^{c}
Canobolas	+	-	81.2^{b}	82.1^{c}	85.8^{ab}	50.6^{a}	53.0^{a}
Canobolas	-	+	81.3^{b}	84.6^{ab}	87.2^{a}	41.2^{b}	51.1^{a}
Canobolas	+	+	84.5^{a}	82.6^{bc}	85.9^{ab}	48.0^{ab}	51.2^{a}
Pooled SEM[4]			0.55	0.41	0.26	7.03	1.10
Source of variation			Significance of treatment effect				
Grain			ns	ns	ns	ns	ns
Xylanase			*	*	*	*	*
Phytase			**	**	**	ns	***
Grain x Xylanase			*	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns
Xylanase x Phytase			**	**	ns	**	**
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-c]Values with unlike superscripts within each column are significantly different at *P <0.05; **P < 0.01; ***P <0.001.
ns = not significant.

The digestibility of gross energy was increased by the inclusion of xylanase (P <0.05) and the inclusion of phytase (P <0.01) and the interaction between the inclusion of xylanase and phytase (P <0.01). Gross energy digestibility was increased by the inclusion of phytase by 9.4

and 9.9 % in the Bogong and Canobolas diets, respectively. Likewise, the digestibility of starch was increased by the inclusion of xylanase (P <0.05) and phytase (P <0.01).

The ileal digestibility of Ca was increased by the inclusion of xylanase (P <0.05) and the interaction (P <0.01) between xylanase and phytase. Similarly, the ileal digestibility of P was significantly increased by the inclusion of xylanase (P <0.05) and phytase (P <0.001), as well as the interaction (P <0.01) between xylanase and phytase. The inclusion of enzymes increased P digestibility by about 13.4 to 29.0% and 30.0 to 35.0%, in the Bogong and Canobolas diets, respectively.

6.3.3 Ileal viscosity and phytate-P content

The results in Table 6.4 show the effect of dietary microbial enzyme supplementation on viscosity of ileal digesta, the content of phytate-P in ileal digesta and phytate degradation in the ileum. In general, the inclusion of enzymes reduced the viscosity and phytate-P content of ileal digesta, as well as increasing the degradation of phytate of the diet.

The inclusion of xylanase and phytase, and the interaction between these two factors reduced (P <0.05) the viscosity of ileal digesta. In addition, the concentration of phytate-P in the ileal digesta was also decreased (P <0.001) by the inclusion of phytase in the diet, the interaction between grain and phytase, as well as the interaction between the three main factors. Furthermore, the interaction between the inclusion of xylanase and phytase simultaneously in the Bogong and Canobolas diets, tended (P =0.092) to have a less value of phytate-P content, comparing to not only the control diets (no enzymes) but also when the diet are added with xylanase only. The degradation of phytate-P at the ileum was numerically higher on the Canobolas than Bogong diet, while it was increased (P <0.05) by the interaction of grain and xylanase, by the inclusion (P <0.001) of xylanase and phytase, and the interaction between xylanase and phytase. The degradation of phytate-P by microbial enzymes was more than two times higher than on the diets without any enzyme supplementation.

Table 6.4 Ileal digesta viscosity, phytate-P content and degradation of phytate of broiler chickens on triticale-based diets with or without enzymes at 21 days of age [1]

Treatments			Viscosity (cP)	Phytate-P (g/kg DMI)	Degradation of phytate (%)
Grain	Xyl[2]	Phy[3]			
Bogong	-	-	3.8[a]	2.5[a]	15.2[d]
Bogong	+	-	2.6[b]	2.4[ab]	32.8[ab]
Bogong	-	+	2.7[b]	2.2[c]	30.7[bc]
Bogong	+	+	2.3[b]	2.0[d]	34.5[a]
Canobolas	-	-	3.9[a]	2.5[ab]	13.1[d]
Canobolas	+	-	2.4[b]	2.4[b]	28.4[c]
Canobolas	-	+	2.7[b]	2.0[d]	31.9[abc]
Canobolas	+	+	2.4[b]	2.2[c]	30.5[bc]
Pooled SEM[4]			0.15	0.03	1.20
Source of variation			Significance of treatment effect		
Grain			ns	ns	*
Xylanase			*	ns	***
Phytase			*	***	***
Grain x Xylanase			ns	***	*
Grain x Phytase			ns	ns	ns
Xylanase x Phytase			*	0.092	***
Grain x Xylanase x Phytase			ns	***	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-d]Values with unlike superscripts within each column are significantly different at $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
ns = not significant.

6.3.4 Relative visceral organ weight

On day 7, there was no statistically significant effect of xylanase and phytase inclusion on the relative weight of any of the organs examined (Table 6.5); however, the relative weight of the proventriculus plus gizzard of birds on the Bogong diets was less ($P < 0.01$) than that on the Canobolas diets. On the other hand, the relative weight of liver of birds on the Bogong diets was higher ($P < 0.01$) than that of birds on the Canobolas diets. The heaviest weight of proventriculus plus gizzard was in birds on the Canobolas diet with phytase inclusion (5.90

g/100 g of body weight), and the lowest weight were observed on birds on the Bogong diet containing phytase (4.30 g/100 g body weight). In addition, there is an interaction (P <0.05) between grain and phytase inclusion to reduce the relative weight of proventriculus and gizzard of birds on the Bogong diet, on the other hand, increase the relative weight of proventriculus and gizzard of birds on the Canobolas diet. The relative weight of lymphoid tissues (spleen and bursa Fabricius) as well as yolk sac were not significantly different (P >0.05).

Table 6.5 Relative weight of visceral organs (g/100g of body weight) of broiler chickens on triticale-based diets with or without enzymes at 7/21 days of age[1]

Treatments			Proventriculus and Gizzard	Small Intestine	Pancreas	Liver	Spleen	Bursa of Fabricius	Yolk sac
Grain	Xyl[2]	Phy[3]							
Bogong	-	-	4.5^{bc}/2.0	9.1/6.9	0.46/0.27	5.0^{ab}/3.5^{ab}	0.07/0.08	0.17/0.20	0.12
Bogong	+	-	4.8^{bc}/1.9	9.2/6.4	0.52/0.27	5.0^{ab}/3.8^{a}	0.08/0.07	0.18/0.17	0.09
Bogong	-	+	4.3^{c}/2.3	9.2/6.5	0.49/0.26	5.2^{a}/2.9^{c}	0.08/0.06	0.18/0.25	0.09
Bogong	+	+	4.4^{bc}/1.9	10.0/6.	0.46/0.26	4.9^{ab}/3.3^{bc}	0.05/0.08	0.21/0.21	0.09
Canobolas	-	-	5.2^{ab}/2.2	9.6/6.9	0.50/0.28	4.5^{bc}/3.8^{a}	0.06/0.12	0.18/0.25	0.03
Canobolas	+	-	4.7^{bc}/2.2	10.1/6.	0.49/0.29	4.6^{bc}/3.7^{a}	0.09/0.09	0.18/0.21	0.02
Canobolas	-	+	5.9^{a}/2.2	10.1/6.	0.50/0.29	4.3^{c}/3.1^{bc}	0.08/0.08	0.20/0.19	0.10
Canobolas	+	+	5.2^{ab}/1.9	8.6/6.3	0.45/0.30	4.5^{bc}/3.2^{bc}	0.08/0.08	0.17/0.19	0.06
Pooled SEM[4]			0.12/0.05	0.16/0.09	0.010/0.006	0.08/0.06	0.003/0.005	0.006/0.008	0.015
Source of variation			Significance of treatment effect						
Grain			**/ns	ns/ns	ns/ns	**/ns	ns/ns	ns/ns	ns
Xylanase			ns/0.086	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns
Phytase			ns/ns	ns/ns	ns/ns	ns/***	ns/ns	ns/ns	ns
Grain x Xylanase			0.08/ns	ns/ns	ns/ns	ns/0.081	ns/ns	ns/ns	ns
Grain x Phytase			**/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns
Xylanase x Phytase			ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns
Grain x Xylanase x Phytase			ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns/ns	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-c]Values with unlike superscripts within each column are significantly different at *P <0.05; **P <0.01; ***P <0.001.
ns = not significant.

On day 21 the only significant effect of the inclusion of enzymes in the diets was on the relative weight of liver, which was decreased (P <0.001) by the inclusion of phytase in the diets. The relative weight of the proventriculus and gizzard tended (P =0.086) to be lower in the diets containing xylanase. The effect of inclusion of enzymes on the relative weight of small intestine was not significant; however, the values on the diet with enzymes were less than those on diets without enzymes. In addition, the inclusion of enzymes did not statistically affect the relative weight of pancreas and immune organs, the spleen and bursa.

6.3.5 Tissue protein content and digestive enzyme activities

In early life (at 7 d), there were no significant of main effects (grain cultivar, supplementary xylanase and phytase) on the pancreas and jejunal tissue protein content and enzyme activities, except for the effect on the xylanase inclusion on the maltase activity in jejunal tissue, which is by inclusion of xylanase in the diet the activity increased (P <0.01) (Table 6.6).

On day 21, tissue protein content and enzyme activities is presented in Table 6.7. There was no significant effect of grain variety, while the inclusion of xylanase significantly decreased (P <0.05) the pancreatic tissue protein content and the inclusion of phytase decreased (P <0.01) the activities chymotrypsin amidase. The activities of jejunal tissue protein, alkaline phosphatase, maltase and sucrase were not significantly (P >0.05) affected by the treatments.

Table 6.6 Tissue protein content and enzyme activities of broiler chickens at day 7, on triticale-based diets with or without microbial enzyme supplementation[1]

Treatments			Pancreas		Jejunum			
Grain	Xyl[2]	Phy[3]	Protein (mg/g tissue)	Chymotrypsin Amidase (ηmol/mg protein/min)	Protein (mg/g tissue)	Alkaline Phosphatase (μmol/mg protein/min)	Maltase (ηmol/mg protein/min)	Sucrase (ηmol/mg protein/min)
Bogong	-	-	175.6	7.0	195.2	5.4	147.1^{c}	27.1
Bogong	+	-	163.8	5.7	183.5	5.4	197.7^{a}	29.7
Bogong	-	+	166.1	6.9	187.3	4.4	153.1^{bc}	30.0
Bogong	+	+	165.1	6.5	185.1	5.2	187.5^{ab}	28.0
Canobolas	-	-	171.1	6.9	207.2	5.4	156.5^{bc}	27.3
Canobolas	+	-	167.4	5.3	182.4	5.7	194.9^{a}	30.2
Canobolas	-	+	169.5	5.4	193.7	4.8	157.6^{bc}	29.5
Canobolas	+	+	164.4	6.1	190.9	5.1	185.6^{ab}	33.3
Pooled SEM[4]			2.180	0.198	4.100	0.212	4.920	0.804
Source of variation			Significance of treatment effect					
Grain			ns	ns	ns	ns	ns	ns
Xylanase			ns	ns	ns	ns	**	ns
Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase			ns	ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.

[2]Xylanase.

[3]Phytase.

[4]SEM = Standard error of mean.

[a-c]Values with unlike superscripts within each column are significantly different at $^{**}P < 0.01$.

ns = not significant.

Table 6.7 Tissue protein content and enzyme activities of broiler chickens at day 21 following rearing on triticale-based diets with or without microbial enzyme supplementation[1]

Treatments			Pancreas		Jejunum			
Grain	Xyl[2]	Phy[3]	Protein (mg/g tissue)	Chymotrypsin Amidase (ηmol/mg protein/min)	Protein (mg/g tissue)	Alkaline Phosphatase (μmol/mg protein/min)	Maltase (ηmol/mg protein/min)	Sucrase
Bogong	-	-	228.9^{ab}	5.8^{a}	250.2	8.9	133.6	18.9
Bogong	+	-	171.5^{c}	4.7^{ab}	199.5	9.9	159.5	23.8
Bogong	-	+	212.1^{abc}	5.6^{a}	232.8	8.1	144.8	21.3
Bogong	+	+	179.3^{c}	4.1^{b}	219.1	8.4	208.8	26.7
Canobolas	-	-	234.6^{a}	5.7^{a}	249.8	10.3	149.9	18.4
Canobolas	+	-	172.4^{c}	5.1^{ab}	155.8	9.8	184.6	26.1
Canobolas	-	+	189.7^{abc}	5.5^{a}	194.4	7.8	140.2	22.0
Canobolas	+	+	184.4b^{c}	3.9^{b}	193.9	8.6	175.1	27.2
Pooled SEM[4]			6.420	0.186	8.35	0.288	6.52	0.920
Source of variation			Significance of treatment effect					
Grain			ns	ns	ns	ns	ns	ns
Xylanase			*	ns	ns	ns	ns	ns
Phytase			ns	**	ns	ns	ns	ns
Grain x Xylanase			ns	ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns	ns	ns
Xylanase x Phytase			0.091	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.

[2]Xylanase.

[3]Phytase.

[4]SEM = Standard error of mean.

[a-c]Values with unlike superscripts within each column are significantly different at *P <0.05; **P <0.01.

ns = not significant.

6.4 DISCUSSION

6.4.1 Gross response

The results of this study demonstrate that supplementation with microbial enzymes improved the performance of broiler chickens in terms of FI, BW and FCR up to 21 days of age. As in the previous experimental work using lower levels of triticale, FI and BW were similar in birds on both diets with that containing only xylanase and in the diet without any enzyme supplementation, whereas diets with only the phytase supplement and diets with the combined phytase and xylanase supplements supported higher FI and BW. This finding is contrary to those of Pettersson and Åman (1988) and Pourreza *et al.* (2007), who showed that supplementation of triticale-based diets with carbohydrase enzymes increased body weight. The feed intake of chickens on a diet based on an 'old' triticale cultivar (Pettersson and Åman, 1988) was more significantly improved by enzyme supplementation than response on 'new' triticale cultivar diet (Pourreza *et al.*, 2007). One of the reasons for the differences may be the lower content of pentosans in the new cultivars of triticale compared to the cultivars used in previous trials. The enzyme (xylanase) appeared to have a bigger impact under higher fibre content. Nevertheless, Oettler (2005) argued that there are many other factors that can affect the nutritional value of triticale used in feeding, such as genotype, growing environment, animal species, feed formula, and methodologies implemented in the experiments.

The highest FI and BW were found in the diets with only phytase inclusion. These diets also exhibited the highest CP, GE, starch and P digestibility. This finding, however, was unexpected, because the ileal viscosity of birds on the diet with only phytase supplementation was significantly higher than that of birds on the diets containing only xylanase or those containing a combination of supplemental xylanase and phytase. This phenomenon may be the result that the microbial phytase used in the present study was produced by solid state fermentation and containing significant activities of beta-glucanase and xylanase (Wu *et al.*, (2004). It might be as effective as xylanase, as or more than xylanase, in improving the performance of broiler chickens fed on triticale diets containing adequate levels of phosphorus. Improved performance with enzyme supplementation was generally associated with reduced digesta viscosity, increased AME, and reduced relative weight small intestine.

The improvement in body weight and feed intake owing to the supplementation of phytase and the combination of supplemental xylanase and phytase is consistent with the findings in

Chapter 5. Except for FCR, these findings are in agreement with the results from wheat-based trials with broiler chickens reported by Ravindran *et al.* (1999b) and Zyla *et al.* (1999), i.e. xylanase and phytase had a synergistic effect with respect to increasing digestibility of energy and nutrients, which contributed to the higher FI, BW and FCR.

Numerically, the diets with only phytase or a combination of supplemental xylanase and phytase had higher nutrient digestibility than diets containing xylanase alone. These results are consistent with the results of phytate degradation, where there is an increase in phytate degradation in the diets with the microbial enzyme preparation compared to the control diet. Phytic acid (phytate-P) is a critical antinutrient present in the grain that can bind minerals, protein, lipids and starch (Thompson and Yoon, 1984), thereby reducing nutrient digestibility in poultry (Sebastian *et al.*, 1997). Other workers have also reported that phytase in broiler chicken diets improved the total amino acid digestibility and ME (Namkung and Leeson, 1999; Ravindran *et al.*, 1999a); however, some of these responses were not assessed in this study. Bedford (1996) described the capacity of xylanase in poultry diet to potentially improve the nutritive value of the diet by hydrolysing polysaccharides which encapsulate the starch or protein. Another advantage in using the various exogenous enzymes is that they can improve the nutritional value of diets by reducing the loss of endogenous material (Dänicke *et al.*, 2000; Selle *et al.*, 2000; Cowieson *et al.*, 2003).

As has been noted above, the results indicate that Bogong and Canobolas have relatively similar nutrient values for broiler chickens, and it can also be said that in triticale-based diet, the activity of one type of enzyme is facilitated by the other, possibly in a complementary way.

6.4.2 Visceral organ weight

In early life of the birds, the relative weight of the proventriculus plus gizzard, duodenum and liver was affected by the cultivars and phytase inclusion. The weight of proventriculus plus gizzard on the Canobolas diet was higher than on the Bogong diet. This may be the result of the fact that Canobolas appears to be slightly coarser than Bogong. In contrast, liver weight of birds on the Bogong diets was higher than that of birds on the Canobolas diets, but the reason for this response is unclear. At day 21, the weight of the pancreas and spleen of birds on the Canobolas diets was higher than for those on Bogong diets. The effect of microbial enzymes could be found on the weight of small intestine (reduced by xylanase inclusion), liver (reduced by phytase inclusion) and bursa of Fabricius (interaction of grain and phytase). The exact

mechanisms for these changes is unknown; however, in general there are three points that can be noted. Firstly, the relative weight of the proventriculus plus gizzard was higher for the Canobolas diets than for the Bogong diets; secondly, the inclusion of xylanase and phytase did not alter the weight of the proventriculus plus gizzard, and thirdly, the inclusion of microbial xylanase and phytase reduced the weight of the small intestine and liver compared with control groups. These results are similar to the results reported by Zarghi *et al.* (2010) who used a high level of triticale in broiler diets. The workers found that the relative weight of the pancreas, small intestine and large intestine decreased with the inclusion of enzyme. The heavier gizzard found in birds on the Canobolas diet compared to the Bogong diet may be as a result of the higher grinding activity required by birds on the Canobolas diet, because the gizzard is stimulated by eating a coarse grain fraction in the diet or ingesting coarse fibre, which leads to a hypertrophy of the organ (Nir *et al.*, 1994; Svihus *et al.*, 1997; Taylor and Jones, 2004). In addition, in the current study, xylanase inclusion lowered the weight of the small intestine. This may be due to diminished physical function of the intestine because a decrease in concentration of water-soluble NSP and subsequent reduction in digesta viscosity could reduce the muscular activity needed to propel the digesta through the tract (Wang *et al.*, 2005).

6.4.3 Tissue protein and digestive enzyme activities

The protein content and the activities of pancreatic protease (chymotrypsin amidase) were higher in birds on the Bogong and Canobolas diets without enzyme inclusion. This may be the result of the need for greater endogenous secretions in order to accomplish digestion. This finding is in agreement with that reported by Mahagna *et al.* (1995) who indicated that a reduction in the secretion of pancreatic chymotrypsin was caused by enzyme supplementation. The authors added that the reduction in secretion of pancreatic enzymes was most probably the result of the presence of exogenous enzymes in the intestine; however, this proposal does not correspond with the results reported by Engberg *et al.* (2004) who found that an increased activity of pancreatic chymotrypsin by the inclusion of xylanase. Xylanase may reduce viscosity and enhance the activity of enzymes that target nutrients other than carbohydrates.

Although it is not affected by the treatments, there was a tendency of reduction in the activity of jejunal alkaline phosphatase activity as a result of the inclusion of microbial enzymes. The activity of the enzyme of birds on the dietary treatments that included phytase was lower than on diets without enzyme, except for the jejunal sucrase activity. This finding is similar to that reported by Huff *et al.* (1998) who proposed that the decrease in alkaline phosphatase

associated with the diets supplemented with phytase might indicate the down regulation of this enzyme in response to the increased availability of P. The activities of jejunal mucosal disaccharidases, as reflected by maltase and sucrase, were significantly affected by the inclusion of xylanase. This result is in agreement with Pinheiro *et al.* (2004) who proposed that supplementation with carbohydrase and protease could increase the activities of sucrase and maltase compared with the response on unsupplemented diets. This may be caused by release of substrates targeted by these enzymes.

6.5 CONCLUSIONS

The response of birds on the diets based solely on triticale was close to or better than breed standard. Supplementation with phytase alone or combination of phytase and xylanase further improved productivity. The beneficial effect of exogenous enzymes may be due to improvement in the digestibility of CP, gross energy, starch, Ca and P. The relative weight of visceral organs especially that of the small intestine, was lower on the diets containing enzymes; however, the weight of the proventriculus plus gizzard differed between the Bogong and Canobolas groups possibly because of intrinsic differences in the coarseness of these two cultivars. The nutritive values of Bogong and Canobolas are similar, and diets based on these cultivars can be improved by the concurrent inclusion of xylanase and phytase. The effects of the treatments on the profiles and activities of microbial populations are described in the next chapter.

CHAPTER 7 RESPONSE OF BROILER CHICKENS TO DIETS BASED ON TRITICALE AND SUPPLEMENTED WITH MICROBIAL ENZYMES. 2. INTESTINAL MICROBIAL PROFILES AND ACTIVITIES

7.1 INTRODUCTION

In the trials conducted by South Australian Research and Development Institute, Bogong and Canobolas were found to be the highest yielding new cultivars of triticale (Crouch and Saunders, 2009). In Chapter 6 it was shown that diets based on these two new cultivars were supported excellent production of broiler chickens. The primary constraint to the use of triticale in poultry diets is the presence of NSP, especially xylans and arabinoxylans, as well as phytate, which together reduce nutrient digestibility.

The inclusion of xylanase and phytase in diets based on Bogong and Canobolas has already been already shown to improve the nutritive value of such diets and improve the productivity of broiler chickens (Chapters 5 and 6). Feed enzymes are known to reduce the bacterial activity in the ileum by reducing the amount of nutrient available for microbial fermentation (Silva and Smithard, 2002). Jamroz *et al.* (2002) suggested that the end-products of microbial fermentation (e.g. SCFA and lactic acid) in the chicken intestine may contribute energy to the host bird. In addition, van der Wielen *et al.* (2000) and Ricke (2003) reported that dietary enzymes may play an important role in regulating the GIT microbial population. The concentrations of SCFA and lactic acid in the GIT depend on the cereal type and feed enzymes used (Jamroz *et al.*, 2002; Silva and Smithard, 2002; Józefiak *et al.*, 2004a; Józefiak *et al.*, 2004b) and reflect to a certain extent the activity of the resident microflora (Engberg *et al.*, 2002).

The aim of this study was to investigate how feeding of diets based on triticale, cultivar Bogong and Canobolas and supplemented with xylanase and phytase influence the gastrointestinal ecosystems of broiler chickens in terms of microbial profiles, concentrations of organic acids and pH.

7.2 MATERIALS AND METHODS

7.2.1 Experimental design and bird management

The samples assessed were drawn from birds and diets described in Chapter 6. The details of the diets offered are presented in Table 6.1. The rearing conditions are fully described in Section 6.2.2. The animal ethics approval and statistical procedures used were described in Section 6.2.4.

On day 21, three birds from each cage, were randomly selected, 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 squeezed and collected in plastic containers and pooled by cage for the determination of nutrient digestibility (described in Chapter 6) and SCFA. Around 1 g of ileal and caecal contents were also collected separately into prepared McCartney bottles containing anaerobic broth (see Appendix A) for the enumeration of microbial populations. The McCartney bottles and plastic containers containing digesta samples for microbial profiles and SCFA concentrations, respectively, were kept at –20ºC until they were analysed.

7.2.2 Measurements and analyses

Gut microbial population

Approximately 1 g of sample of either ileal or caecal digesta was placed into a pre-weighed McCartney bottle containing 8 mL of anaerobic broth and 1 mL of reducing (R) solution (see Appendices A and C for the preparation). After addition of sample to the anaerobic broth, the bottle with broth and sample was weighed again. Once the sample was weighed, the first dilution of the sample was calculated by subtracting the weight of the bottle and the broth plus the R solution from the final weight of the bottle containing the sample (this is the sample weight), and dividing the sample weight by the broth plus R solution. This calculation yield is the proportion of the original sample per mL of solution in the bottle.

The suspension was homogenised 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 Miller and Wolin (1974) and Engberg *et al.* (2004). A 1 mL aliquot of the homogenised 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 of each sample was plated on the appropriate medium (10 mL) for enumeration of microbial populations.

Total anaerobic bacteria were grown in anaerobic roll tubes containing 3 mL of Wilkins-Chalgren anaerobic agar (Oxoid, CM 0619) incubated at 39ºC for 7 days. Lactic acid bacteria were enumerated after incubation on MRS agar (Oxoid, CM0361) under anaerobic conditions at 39ºC for 48 h. Lactobacilli were enumerated on Rogossa agar (Oxoid, CM 0627) after anaerobic incubation at 39ºC for 48 h. Enterobacteria were incubated on MacConkey agar (Oxoid, CM 0007) aerobically at 39ºC for 24 h prior to counting. *Clostridium perfringens* (Cp) colonies 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 39ºC 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. After incubation, colonies formed on the respective media were carefully counted, converted into logarithmic equivalents (log_{10}) and expressed as numbers of colony-forming units (CFU) per gram of wet ileal or caecal digesta.

Measurement of organic acids

A modification of the analytical method described by Jensen *et al.* (1995) for the analysis of organic acids (SCFAs, lactic acid and succinic acid) concentrations was used. In general, frozen ileal and caecal samples were thawed and homogenised by vigorous shaking. About 1 to 2 g of ileal and caecal digesta (wet weight) were accurately weighed into centrifuge tubes (placed on ice) and 1 mL internal standard (0.01 methylbutyric acid) was added and thoroughly mixed with a vortex mixer, followed by centrifugation at 25,700 × **g** at 5ºC for 20 min in a Beckman model J2-21M Induction Drive Centrifuge with a JA-21 rotor. The supernatant (approximately 1 mL) was transferred into 8-mL vials (on ice). At this stage, the standards and blanks were prepared. One mL of the standard acid mixture was accurately transferred to the 8-mL vials. The blank was treated similarly (1 mL of Milli-Q water), and 0.1 mL of 0.1 M ethylbutyric acid was added to the standards and blank vials. Two mL of ether and 0.5 mL of concentrated HCl (36%) were then added to all the samples, the standard and the blank and the lids were tightened prior to vortexing for about 1 minute. After repeat vortexing, all the tubes were centrifuged at 2060 × **g** for 15 min at 5ºC. Accurately, 400 µL of the supernatant were

transferred into gas chromatograph (GC) vials (2 mL) and mixed with 40 μL of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) or 50 μL MTBSTFA for the standard. The GC vials were tightly capped, vortexed and kept on a heating block at 80ºC for 20 min and then left at room temperature for 48 h. After 48 h, concentrations of the different organic acids were determined, using a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA).

Intestinal pH

The pH of ileal and caecal digesta was measured on fresh samples collected at 21 d of age. The pH was determined by the modified procedure of Corrier *et al.* (1990). Around 1 g of content was diluted in 9 mL of cold distilled MilliQ water. The suspension was mixed thoroughly with a stirrer and the pH was determined by insertion of a glass electrode (EcoScan 5/6 pH meter, Eutech Instruments Pte Ltd., Singapore).

7.3 RESULTS

7.3.1 Gut microflora

The populations of total aerobic bacteria (TAB), lactic acid bacteria (LAB), lactobacilli and C. *perfringens* in the ileal digesta were not significantly affected (P >0.05) by grain cultivar, inclusion of xylanase or phytase. However, the population of enterobacteria was increased (P <0.05) by the inclusion of phytase. An interaction between grain cultivar, xylanase and phytase was significant (P <0.01) in the case of the population of LAB, tending to rise with the inclusion of both enzymes (Table 7.1). A grain × xylanase interaction (P <0.01) was noticed on *C. perfringens* population, which in Canobolas-based diet was higher than in Bogong-based diet. Furthermore, there was an interaction (P = 0.067) between xylanase and phytase on the population of *C. perfringens*, although there were opposite trends in Bogong- and Canobolas-based diets. In Bogong-based diets, the population of *C. perfringens* was less than on the control diet while it was higher in Canobolas-based diet.

Table 7.1 Bacterial counts (log_{10} CFU/g digesta) in ileal digesta of broiler chickens triticale-based diets with or without enzyme supplementation[1]

Treatments							
Grain	Xyl[2]	Phy[3]	Total Anaerobic	Lactic acid	Lactobacilli	Entero-bacteria	*C. perfringens*
Bogong	-	-	7.1	8.0^{abc}	7.1	4.6	4.3^{abc}
Bogong	+	-	7.1	8.2^{ab}	7.3	4.7	4.3^{abc}
Bogong	-	+	7.0	8.1^{abc}	7.1	4.8	4.5^{ab}
Bogong	+	+	7.1	7.8^{bc}	7.1	4.8	3.9^{c}
Canobolas	-	-	7.1	8.5^{a}	7.1	4.6	4.1^{bc}
Canobolas	+	-	7.1	7.6^{c}	7.1	4.8	4.8^{a}
Canobolas	-	+	7.0	8.2^{ab}	7.1	4.8	4.2^{bc}
Canobolas	+	+	7.0	8.4^{a}	7.1	5.0	4.5^{ab}
Pooled SEM[4]			0.08	0.07	0.08	0.04	0.07
Source of variation			Significance of treatment effect				
Grain			ns	ns	ns	ns	ns
Xylanase			ns	ns	ns	ns	ns
Phytase			ns	ns	ns	*	ns
Grain x Xylanase			ns	ns	ns	ns	**
Grain x Phytase			ns	ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns	0.067
Grain x Xylanase x Phytase			ns	**	ns	ns	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-c]Values with unlike superscripts within each column are significantly different at $^{**}P < 0.01$.
ns = not significant.

In the caecal digesta, the grain, xylanase and the phytase inclusion did not affect the population of TAB, LAB, lactobacilli and enterobacteria (Table 7.2). Meanwhile, the inclusion of xylanase decreased ($P < 0.01$). An interaction between grain and phytase was noticed in the population of enterobacteria ($P < 0.05$) and *C. perfringens* ($P = 0.055$). The population of enterobacteria was less in Bogong diet plus phytase than the Bogong diet without enzyme, however; it was opposite in the Canobolas diet, which the Canobolas diet plus phytate had higher population of enterobacteria than the Canobolas diet without enzyme. An interaction ($P < 0.01$) between

grain, xylanase and phytase inclusion indicated that the population of enterobacteria that in Bogong diet decreased the population, on the contrary, it increased in Canobolas diet. An interaction (P =0.074) between grain, xylanase and phytase inclusion also noticed in the population of *C. perfringens*, which decreased in both diets with the inclusion of xylanase and phytase.

Table 7.2 Bacterial counts (log10 CFU/g digesta) in caecal digesta of broiler chickens fed triticale-based diets with or without enzyme supplementation[1]

Treatments							
Grain	Xyl[2]	Phy[3]	Total Anaerobic	Lactic acid	Lactobacilli	Entero-bacteria	*C. perfringens*
Bogong	-	-	8.8	9.4	7.8	8.1^{a}	6.1^{a}
Bogong	+	-	8.8	9.3	7.7	8.0^{abc}	5.8^{bc}
Bogong	-	+	8.7	9.4	7.9	7.8^{bcd}	6.0^{ab}
Bogong	+	+	8.7	9.3	7.9	7.8^{abcd}	5.9^{abc}
Canobolas	-	-	8.7	9.4	7.7	7.7^{d}	6.1^{a}
Canobolas	+	-	8.7	9.4	7.9	8.1^{a}	6.0^{a}
Canobolas	-	+	8.7	9.3	7.8	8.0^{ab}	6.0^{a}
Canobolas	+	+	8.7	9.3	7.8	7.8^{cd}	5.7^{c}
Pooled SEM[4]			0.05	0.04	0.04	0.04	0.03
Source of variation			Significance of treatment effect				
Grain			ns	ns	ns	ns	ns
Xylanase			ns	ns	ns	ns	**
Phytase			ns	ns	ns	ns	ns
Grain x Xylanase			ns	ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	*	0.055
Xylanase x Phytase			ns	ns	ns	0.066	ns
Grain x Xylanase x Phytase			ns	ns	ns	**	0.074

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-d]Values with unlike superscripts within each column are significantly different at *P <0.05; **P <0.01.
ns = not significant.

7.3.2 Short-chain fatty acids, lactic acid and succinic acid

The concentrations of formic, acetic, butyric, lactic, succinic acids and lactic acid of ileal are shown in Table 7.3. There was no significant difference in concentrations of formic, lactic and succinic acids, but acetic acid concentration decreased (P <0.01) in birds on diets supplemented with phytase and was numerically reduced (P =0.066) by the inclusion of xylanase in both Bogong- and Canobolas-based diets.

Table 7.3 Concentrations of various short-chain acids (µmol/g wet digesta) in ileal contents of broiler chickens on triticale-based diets with or without enzyme supplementation[1]

Treatments						
Grain	Xyl[2]	Phy[3]	Formic	Acetic	Lactic	Succinic
Bogong	-	-	0.31	1.6^{ab}	22.6	0.35
Bogong	+	-	0.34	1.4^{abc}	17.1	0.24
Bogong	-	+	0.31	1.2^{bc}	16.0	0.83
Bogong	+	+	0.31	1.0^{c}	19.2	0.44
Canobolas	-	-	0.43	1.8^{a}	15.3	0.19
Canobolas	+	-	0.30	1.4^{abc}	11.9	0.34
Canobolas	-	+	0.37	1.2^{bc}	12.4	0.35
Canobolas	+	+	0.25	0.9^{c}	13.8	0.11
Pooled SEM[4]			0.015	0.08	1.08	0.07
Source of variation			Significance of treatment effect			
Grain			ns	ns	ns	ns
Xylanase			ns	0.066	ns	ns
Phytase			ns	**	ns	ns
Grain x Xylanase			ns	ns	ns	ns
Grain x Phytase			ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-c]Values with unlike superscripts within each column are significantly different at **P <0.01.
ns = not significant.

In the caecal contents, the organic acids recovered were acetic, propionic, butyric and isobutyric, valeric and isovaleric, as well as lactic and succinic acids (Table 7.4). The grain factor affected (P <0.01) the concentration of propionic, butyric plus isobutyric and lactic acid, which the concentration of that acids was higher in Bogong diets than in Canobolas diets. In addition, the concentration of valeric plus isovaleric acid was higher (P <0.05) in Bogong diets than in Canobolas diets. The inclusion of xylanase in both diets significantly increased acetic acid concentration (P <0.05) and butyric plus isobutyric concentration (P <0.01). However, all the organic acids were not affected (P >0.05) by the inclusion of phytase.

A grain × xylanase interaction (P <0.05) indicated that xylanase inclusion increased acetic acid and lactic acid concentration in Bogong diets but decreased in Canobolas diets. Meanwhile, the interaction between grain and xylanase (P =0.081) and (P =0.58) affected the concentration of propionic acid and valeric plus isovaleric acid, respectively. In addition, an interaction (P <0.05) between grain and phytase inclusion was noticed in the concentration of propionic acid, which in Bogong diet with phytase was higher than Bogong diet without enzyme, on the other hand, in Canobolas diet plus phytase, the concentration was less than the Canobolas diet without enzyme.

Table 7.4 Concentration of various organic and mineral acids (µmol/g wet digesta) in caecal contents of broiler chickens on high-yielding triticale-based diet with and without enzymes[1]

Treatments								
Grain	Xyl[2]	Phy[3]	Acetic	Propionic	Butyric + Isobutyric	Valeric + Isovaleric	Lactic	Succinic
Bogong	-	-	76.2^{c}	7.2^{bc}	19.8^{bc}	1.6^{b}	9.1^{ab}	25.2
Bogong	+	-	133.3^{ab}	6.4^{bc}	30.8^{ab}	1.8^{ab}	19.4^{a}	38.5
Bogong	-	+	104.2^{bc}	9.8^{ab}	21.7^{bc}	2.0^{ab}	4.7^{b}	34.8
Bogong	+	+	169.3^{a}	12.5^{a}	37.2^{a}	2.7^{a}	18.7^{a}	51.2
Canobolas	-	-	103.8^{bc}	7.4^{bc}	13.4^{c}	1.6^{b}	7.4^{ab}	52.5
Canobolas	+	-	107.5^{bc}	4.6^{c}	18.6^{c}	1.1^{b}	1.2^{b}	30.5
Canobolas	-	+	100.6^{bc}	6.9^{bc}	17.9^{c}	1.6^{b}	2.0^{b}	33.7
Canobolas	+	+	98.2^{bc}	3.7^{c}	20.2^{bc}	1.2^{b}	1.2^{b}	35.7
Pooled SEM[4]			7.09	0.63	1.74	0.13	1.72	3.54
Source of variation			Significance of treatment effect					
Grain			ns	**	**	*	**	ns
Xylanase			*	ns	**	ns	ns	ns
Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase			*	0.081	ns	0.058	*	ns
Grain x Phytase			ns	*	ns	ns	ns	ns
Xylanase x Phytase			ns	ns	ns	ns	ns	ns
Grain x Xylanase x Phytase			ns	ns	ns	ns	ns	ns

[1]Each value represents the mean of 6 replicates.
[2]Xylanase.
[3]Phytase.
[4]SEM = Standard error of mean.
[a-c]Values with unlike superscripts within each column are significantly different at **P* <0.05; ***P* <0.01.
ns = not significant.

7.3.3 Intestinal pH

There were no significant effects of grain, the inclusion of xylanase and phytase or interactions between these factors for both ileal and caecal pH (Figure 7.1); however, the pH of the caecal content tended (*P* =0.07) to be higher on Canobolas than Bogong-based diets. Furthermore, the caecal pH was numerically reduced by the inclusion of xylanase as well as the combination of supplemental xylanase and phytase.

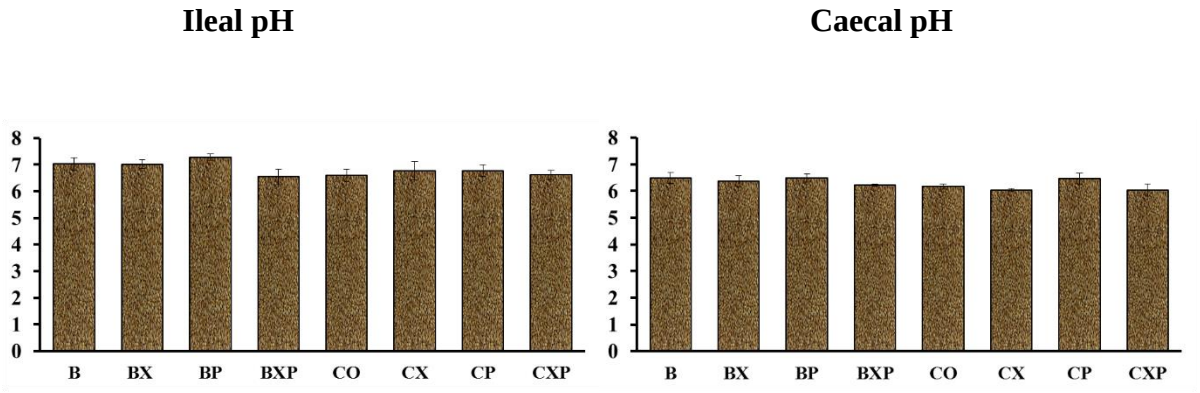

Figure 7.1 pH of ileal and caecal contents of broiler chickens on triticale-based diets with or without enzymes supplementation.

7.4 DISCUSSION

7.4.1 Microbial profiles and activities

In this study the counts of the culture-based bacterial species were partly affected by the dietary treatments as well as the difference in the triticale cultivars and by enzyme supplementation. Populations of TAB and lactobacilli in both the ileum and caeca, and LAB in the caeca were not affected by the dietary treatments. In general, the microbial population at 21 d mirrored the relative concentrations of organic acids, which was dominated by lactic and acetic acids in the GIT of birds on both the Bogong and Canobolas diets; however, the concentration of lactic acid was affected by the grain used and it is likely that the supplemental enzymes stimulated the growth of lactic acid bacteria. This occurred on the Bogong diets, on which birds recorded a higher lactic acid concentration than on Canobolas diets. This result corresponds to the result of Engberg *et al.* (2004) who reported that lactic acid bacteria in the small intestine are stimulated by inclusion of xylanase; however, the population of lactobacilli in the ileum was not affected by the dietary treatment although the number of colonies was higher in birds on the diet that included xylanase. This finding is similar to that reported by Vahjen *et al.* (1998). High concentration of lactic acid and lactobacilli counts in the small intestine indicate that xylanase may have already fermented some of the carbohydrate in this region, but some sugars that have not been digested will enter the caeca and be fermented by the caecal microflora (Choct *et al.*, 1999b; Bedford, 2000). The high SCFA concentration in caecal content and the significantly lower pH owing to the xylanase inclusion helps to explain this finding.

Although SCFA produced in the caeca can yield only relatively small amounts of energy, they can provide other benefits. It has been reported that high fermentation activity in the caeca of chickens correlates with a lower pH, which has the potential to inhibit some pathogenic bacteria (Russell, 1992; van der Wielen *et al.*, 2000). In addition, McHan and Shotts (1993) reported the toxic effect of SCFA on some enterobacteria such as *Salmonella typhimurium*. Using *in vitro* technique, they showed that the presence of SCFA could reduce the numbers of these bacteria by up to 50–80%. In addition, the presence of certain SCFA in the chicken gut, such as lactic and acetic acids, have been associated with a decrease in the survival and adherence of *Salmonella enterica*, *E. coli* and *C. perfringens* (Engberg *et al.*, 2002; Engberg *et al.*, 2004; Bjerrum *et al.*, 2005). The lower number of enterobacteria colonies is also related to the high concentration of lactic acid. Józefiak *et al.* (2004b) reported that lactic acid is the main by-product of carbohydrate fermentation, which is produced by lactic acid bacteria. Lactic acid has been found to be important to broiler gut health and human food safety because its presence can inhibit enterobacteria (Bjerrum *et al.*, 2005). In this study, it was found that the population of enterobacteria and *C. perfringens,* which are considered to be risk groups, is generally smaller than that of lactobacilli that are regarded as beneficial bacteria; however, the increased population of enterobacteria and *C. perfringens* was undoubtedly affected by the supplemental enzymes on the diets. There were no symptoms of necrotic enteritis in this experiment that could be connected to the population of *C. perfringens.* Moreover, the mortality on this trial was negligible, less than 1% and occurring in the first 7 d with no indications of infectious diseases.

7.5 CONCLUSIONS

The lactic acid bacteria and *C. perfringens* in the ileum and enterobacteria and *C. perfringens* in the caeca were significantly affected by the dietary treatments. Furthermore concentrations of organic acids in the digesta were affected by grain type and enzyme supplementation. The population of gram negative bacterial in the digesta was significantly reduced by the inclusion of xylanase and the combination of xylanase and phytase; however, the values were higher on the Bogong-based diets than on the Canobolas-based diets. It is not known if this difference contributed in any way to the difference in performance.

CHAPTER 8 GENERAL DISCUSSION

8.1 INTRODUCTION

Feed costs are a major component of the total production cost in the commercial poultry industry. The poultry industry generally relies on some major ingredients for feed formulation. Cereal grains are the key ingredients in poultry diets, included mainly to provide energy, but grains also provide essential amino acids and other nutrients. Globally, maize is the most commonly used cereal grain in poultry diets but significant amounts of wheat, sorghum, barley and rice or rice by-products are also used when their prices and availability allow for their inclusion. In order to maximise profit, the industry is inclined to use the cheapest ingredients (Batal, 2009). As has been reviewed in Chapter 2, the use of other cereals in poultry diets, including oats, rye, triticale and millet is relatively minor. Limitations to the use of such cereals include the presence of anti-nutritional factors as well as the variation in nutritive value owing to genetic redevelopment, different agronomic conditions and harvest time.

Triticale was the first human-made grain, resulting from crosses between wheat and rye back in 1875. Initially, the cultivars that were developed combined the height and bread quality requirement from wheat and the winter-hardiness and disease resistance from rye (Merker, 1971; Gregory, 1973; Larter, 1973; Sanchez-Monge, 1973; Mackowiak and Lapinski, 1985); however, triticale is currently not used on a large scale in the baking industry. Its softness in texture, which is acquired from rye, makes triticale dough not as hard as wheat. The lower content of amylose in triticale than in wheat, as reported in Chapter 3, is one of the main reasons. In Australia, triticale is primarily used as a feed grain by the livestock industries, and only a small portion is used as food for human consumption. All Australian triticale is produced for domestic use, unlike wheat, barley, oats and sorghum production, some of which are exported. Triticale is a viable feed resource for poultry, pigs and ruminants that may be used in grain, forage or silage form. It has been used in poultry diets for more than half a decade (Sell *et al.*, 1962).

Breeding efforts to develop new cultivars of triticale by combining the positive traits from wheat and rye are still under way. The project reported in this thesis was undertaken to enable the evaluation of new cultivars of triticale developed by the breeding group located at the University of New England, Armidale.

The ME content of triticale in high-yielding cultivars was relatively adequate between harvest years and cultivars (Chapter 3). When compared to wheat, the higher lysine as well as total protein contents of triticale have made it a crop of interest for poultry feeding. Furthermore, starch, amylose and amylopectin, crude fat, mineral and amino acid concentrations, *in vitro* digestibility of DM as well as the viscosity of the digesta from birds ingesting triticale-based diets were also consistent between two different harvest years. In addition, phytate-P and soluble NSP contents were also similar between the cultivars and in between two harvest years. These anti-nutritional factors might still be a limitation for poultry, however phytate-P in the new cultivars was lower than in old cultivars reported by Singh and Sedeh (1979) as well as the fact that soluble NSP content was lower than that of maize and soybean meal reported by Meng and Slominski (2005). The similarity of these nutritional characteristics especially between years, relative stability of the genotype and ease of inclusion in dietary formulation make these varieties suitable candidates for animal feeding.

8.2 PRODUCTIVITY OF BROILER CHICKENS ON TRITICALE-BASED DIETS WITHOUT SUPPLEMENTARY MICROBIAL ENZYME

Feed intake of broiler chickens in early life was not affected by the differences in the diets containing five different triticale cultivars, maize or wheat. This is possibly the result of the dependence on residual yolk sac nutrients for the first four days post hatch (Turro *et al.*, 1994; Malik *et al.*, 2011). By 21 d birds fed Bogong, Jackie, Endeavour and maize diets were superior to those on the other diets; however, the body weight achieved on day 21 was lower than the Cobb Breeder specification. This may be due to lower starting weight, which was about 15% less than the guide. Moreover, the diets were also fed without microbial enzyme supplements and this is most obvious on the wheat-based diet, which has the highest content of arabinoxylans, soluble NSP that are known to be anti-nutritional factor for poultry. This fact supported the findings that anti-nutritional factors can inhibit the availability of nutrients. The feed intake and body weight of the birds offered triticale-based diets were similar to those on the maize-based diets and higher than the wheat-based group, which may be the result of the availability of higher nutrient on the triticale diets relative to the wheat diet.

8.3 ENERGY UTILISATION BY BIRDS ON TEST DIETS

There were two trials to assess the energy utilisation of broiler chickens offered diets based on different grain sources. In the first trial, five high-yielding triticale cultivars (Bogong, Canobolas, Jackie, Tobruk and Endeavour) were compared with maize and wheat diets without enzyme supplementation (Chapter 4). The second trial focussed on two cultivars; Bogong and Canobolas, which partially substituted for maize in diets, with the supplementation of xylanase and phytase (Chapter 5).

The determined ME ranged from 11.4 (Endeavour diet) to 13.0 MJ (Bogong diet), while the maize and wheat diets were 12.8 and 13.3 MJ/kg, respectively. The ME intake of birds on Canobolas and wheat-based diets was low (9.5 and 9.4 MJ, respectively) compared with the other diets, which ranged from 10.2 to 13.6 MJ. These differences may be the result of fibre content and anti-nutritive factors present in the diets, as outlined by Barteczko *et al.* (2009), soluble NSP have a negative relationship with AME. Anaerobic microbes in the small intestine may also influence the degradation of dietary components, particularly NSP (Choct *et al.*, 1996) and the digestibility of nutrients (Steenfeldt *et al.*, 1995; Smits *et al.*, 1997). The difference in ME, energy, fat and protein intake between the triticale-based diets may be the result of the difference in digestibility between the triticale cultivars; however, from the results in Chapter 3, these differences were minimal. The net energy of production (NEp) was different between the triticale, wheat and maize-based diets. Bogong, Jackie and Tobruk diets were comparable with the maize diet, which was higher in NEp than the wheat diet, indicating that these three cultivars of triticale can supply more energy for production and maintenance; however, the higher NEp was also associated with higher HP on such diets. Bogong, Jackie and Tobruk groups also showed higher energy retention as fat and protein than birds fed on the maize and wheat groups; however, the efficiency of ME utilisation for energy and protein retention was not different between the diets. On the other hand, ME utilisation for fat on Bogong, Canobolas, Jackie and Tobruk was higher than values on the maize and wheat diets. An increase in energy:protein ratio in the diet may stimulate a higher deposition of fat (Wiseman and Lewis, 1998), the higher fat deposition of the carcass of the birds fed Bogong, Jackie and Tobruk-based diets may therefore be the results of the higher digestibility of energy as well as protein, because all the diets were formulated to be isocaloric and isonitrogenous.

Supplementation of xylanase or the combination of xylanase and phytase did not significantly affect energy utilisation in diets in which Bogong and Canobolas substituted for maize. The

value of ME and the intakes of ME, GE, fat and protein was slightly increased by the inclusion of xylanase in all diets. Fat intake was significantly higher on the maize diets, followed by Canobolas and Bogong-substituted diets. The values of fat and protein intake were numerically higher in the all-maize diet and substituted-triticale diets. These intakes might be related to the fat and crude protein contents of the constituent ingredients. Maize has higher fat than triticale; on the other hand, the protein content of triticale is higher than that of maize. The NEp and HP tended to increase with inclusion of xylanase, but decreased slightly when a combination of supplemental xylanase and phytase was used. This was also the situation with ME value, the intakes of ME, GE, fat and protein, which were numerically reduced when phytase was added, while xylanase tended to increase the values. The mechanism by which phytase reduced these energy utilisation components was unclear. Xylanase in the diet can increase the values when it stimulates degradation of NSP in the diet and consequently improve the utilisation of energy and nutrients.

8.4 NUTRIENT DIGESTIBILITY

The birds fed on triticale-based diets generally performed at the same level as those on maize and responded positively to supplementation with combined xylanase and phytase. These findings contrast with the pattern of energy utilisation, reviewed previously, which was not improved by the combination of supplemental xylanase and phytase (Chapter 5). A further investigation was conducted as reported in Chapter 6, in which body weight, feed intake and FCR were improved in birds provided with diets in which triticale completely replaced maize and with enzyme supplementation (xylanase and phytase individually and in combination). The gross response results corresponded with the results of ileal digestibility. Apparent ileal digestibility of crude protein, gross energy, starch, Ca and P were increased by enzyme inclusion. The inclusion of phytase alone or the combination of xylanase and phytase had a higher energy and nutrient digestibility than on the diets without enzyme inclusion or the diets with the inclusion of xylanase alone, and it was manifested in higher feed intake, body weight and FCR on the birds ingesting diets with the inclusion of phytase alone or the combination of xylanase and phytase than the birds fed diets without enzyme inclusion or the diets with the inclusion of xylanase alone. The increase in dietary protein digestibility owing to the presence of phytase is caused by the enzyme destroying the binary protein-phytate complexes in the upper digestive tract before hydrolysing the phytate (Selle *et al.*, 2000). The inclusion of phytase released some of the phytate-bound P, which is demonstrated by the higher degradation

of phytate (Chapter 6), leading to the increased ileal digestibility of Ca and P. Although the digestibility of amino acids was not assessed, it can be assumed that it would have a similar trend with the digestibility of other nutrients. Ravindran *et al.* (1999a) postulated that phytate may also increase endogenous amino acid losses and that part of the improvements in apparent amino acid digestibility following phytase addition may arise from reduced endogenous losses. There is also some synergy in amino acid digestibility following the combined inclusion of xylanase and phytase (Ravindran *et al.*, 1999b; Selle *et al.*, 2003). The ileal viscosity of birds on the diet with only phytase supplementation was numerically higher than that of birds on the diet containing xylanase and a combination of supplemental xylanase and phytase. This effect of phytase on viscosity is unclear.

The heavier gizzard found in birds on the Canobolas diet compared with Bogong diet may be as a result of the higher grinding activity required by birds on the Canobolas diet, because the gizzard is stimulated by eating a coarse grain fraction in the diet or ingesting coarse fibre, which leads to a hypertrophy of the organ (Nir *et al.*, 1994; Svihus *et al.*, 1997; Taylor and Jones, 2004). In addition, in the current study, xylanase inclusion lowered the weight of the small intestine. This may be the result of diminished physical function of these parts, because a decrease in water-soluble NSP and subsequent reduction in digesta viscosity induce a feedback mechanism in gut motility and thus a decrease in the size of this organ (Wang *et al.*, 2005).

The presence of exogenous enzymes in the intestine resulted in a reduction in tissue protein of pancreas and pancreatic chymotrypsin as well as the activity of jejunal alkaline phosphatase. The activities of jejunal mucosal disaccharidases, maltase and sucrase, were significantly increased by the inclusion of xylanase. The high activity of maltase and sucrase is probably related to an increase in target substrates, maltose and sucrose, in the jejunum of the birds. Supplementation with carbohydrase and protease has been shown to increase the activities of sucrase and maltase (Pinheiro *et al.*, 2004). This may be caused by the release of substrates targeted by these enzymes.

The total anaerobic and lactobacilli bacteria in both ileum and caeca were not affected by the dietary treatments (Chapter 7). The relative concentrations of organic acids were dominated by the presence of lactic and acetic acids; however, the concentration of lactic acid was not significantly different between the cultivars used, being higher on the Bogong- than Canobolas-based diets. It is likely that the supplementary enzymes stimulated the growth of lactic acid bacteria more effectively in Bogong-based diet. The high counts of lactic acid bacteria and

lactobacilli in the small intestine may indicate that an increase in hydrolytic activity owing to xylanase although some sugars would remain undigested enter the caeca, to be fermented by the caecal microflora (Choct *et al.*, 1999b; Bedford, 2000). On the enzyme supplemented diets, especially xylanase in Bogong-based diet, these resulted in a higher organic acid concentration in the caeca, which might relate to the higher body weight in those groups. There was a decrease in the population of total anaerobic bacteria, enterobacteria and *C. perfringens*, which are considered to be risk groups, possibly in response to the relatively high concentration of organic acid (Gunal *et al.*, 2006). However the results for the Bogong-based diets were slightly in contrast with those on the Canobolas-based diets, there was no incidence of necrotic enteritis in this experiment owing to the high population of *C. perfringens* in the Canobolas group.

8.5 CONCLUSION AND RECOMMENDATIONS

The current studies clearly demonstrated the potential of high-yielding triticale cultivars to become important ingredients in poultry diets. An overview of the results obtained from the laboratory study and three feeding trials demonstrate the performance of broiler birds in terms of body weight, feed intake and FCR comparable to that of maize or wheat-based diets. The potential of the triticale cultivars also lies in low variability between the cultivars as well as the similarity in nutritive value between the harvest years making it easier to interchangeably include them in diet formulation.

Without enzyme supplementation, the nutritive and energy value of triticale cultivars in broiler chicken diet were relatively low and dependant on cultivar. Bogong was the best-performing cultivar in a balanced broiler grower diet, providing for slightly better performance than maize while Canobolas supported the lowest performance, similar to wheat. A combination of supplemental xylanase and phytase in the Bogong and Canobolas-based diets did not appreciably improve performance of birds. Furthermore, the similarity in results from birds on both Bogong and Canobolas-based diets was mirrored in their nutrient digestibility, ileal viscosity, phytate-P degradation, relative organ weight, tissue protein and digestive enzyme activities, and microbial profiles and activities.

In order to have comprehensive information on the nutritive value of the test cultivars for poultry and the effectiveness of enzyme supplementation of diets containing these grains, future research should be directed to the following areas:

- Gut histology of broiler chickens, to elucidate any changes in mucosal morphometry and digestive function;
- The effect of grain processing, including pelleting characteristics, particle size and other commercial procedures such as extrusion, and
- Assessment of the potential benefit of a wider range of enzyme products that have carbohydrase, protease and phytase activities.

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APPENDICES

Appendix A Composition of anaerobic broth (per litre)

No	Compound	Amount
1	Yeast extracts	2.5 g
2	Peptone from casein	2.5 g
3	Solution A	167 mL
4	Solution B	167 mL
5	Resazurin solution	1 mL
6	Hemine solution	5 mL
7	Tween 80	1 mL
8	Milli-Q water	Make up to 1 litre

Preparation:

Solution A, solution B, Resazurin solution and Hemine solution are firstly prepared and stored in each different labelled bottle (see Appendix B). Yeast extract (2.5 g) and peptone (2.5 g) are added to approximately 200 mL water and mixed with a stirring magnet on a stirring plate. Solution A (167 mL) and solution B (167 mL), 1 mL resazurin solution, 5 mL hemine solution and 1 mL Tween 80 are added. This solution is bubbled with CO_2 for 20 min. It is then autoclaved for 15 min, until the solution turns yellow. If the sample will be in storage for a long period, the 50% water should be replaced with glycerol, but the amount of solids should remain the same.

After autoclaving, the solution is cooled at room temperature while being bubbled with CO_2. The CO_2 flushing id continued as the solution is distributed into tubes (8 mL) or bottles (10 mL) prior to autoclaving.

Appendix B Chemical composition of Solution A, Solution B, Resazurin solution and Hemine solution

No	Compound	Amount
1	Solution A (in 1 L Milli-Q water)	
	$NaCl$	5.4 g
	KH_2PO_4	2.7 g
	$CaCl_2.H_2O$	0.16 g
	$MgCl_2.6H_2O$	0.12 g
	$MnSO_4.\ 4H_2O$	0.07 g
	$CoCl_2.6H_2O$	0.06 g
	$(NH_4)_2SO_4$	5.4 g
	$FeSO_4.7H_2O$	0.05 g
2	Solution B (in 1 L Milli-Q water)	
	$K_2HPO_4.3H_2O$	2.7 g
3	Resazurin solution (in 100 mL Milli-Q water)	
	Resazurin	100 mg
4	Hemine solution (in 100 mL 0.02% NaOH)	

Appendix C Composition of R solution (per litre)

No	Item	Amount
1	$NaHCO_3$	50 g
2	L-Cysteine hydrochloride	2.5 g
3	$Na_2S.9H_2O$	2.5 g
4	Milli-Q water	1 L

Preparation:

R-solution (Reducing solution) is prepared under a flow of N_2. One litre of Milli-Q water is placed on a stirring plate and mixed with a stirring magnet while being bubbled with N_2. During stirring, the compounds are added one at time. After all the solids have dissolved, the solution is poured into rubber- and screw-cap bottles. All the bottles are then bubbled again with N_2 and then secured with the rubber stopper and screw cap. Finally, all the bottles are autoclaved at 110ºC at approximately 105 kPa for 15 min.