Nutrition and Nutritional Value of Wheat Grown in Organic and Conventional Farming Systems in South Australia

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Abstract

With the growth of the organic industry world wide, unsubstantiated claims have been made that organic produce is of higher nutritional value than conventionally grown produce. The aim of this study was to test these claims by comparing nutritional value of organically and conventionally grown wheat.

Paired 'across the fence' field comparisons were made (a total of fourteen over two seasons) between certified organic farms and conventional neighbours in three South Australian locations. The organic and conventional treatments mirrored, where possible, the management practices of the organic and conventional farmers so that the grain produced in the comparisons accurately represented grain produced and marketed as either organically or conventionally grown wheat.

The grain was analysed for elemental and amino acid composition. Differences included lower concentrations of phosphorus (hence lower phytate), potassium, magnesium and manganese and higher concentrations of zinc and copper in organically grown grain. There were no consistent differences in amino acid composition.

Due to the known antagonism of phytate on intestinal zinc absorption, these compositional differences were hypothesised to be additive in increasing zinc nutritional value of organically grown wheat. This hypothesis was tested using a rat model.

Four diets were formulated based on wheat from the field trials: organic (ORG), organic with added phytate (ORG+PHYT), conventional (CONV) and conventional with added zinc (CONV+Zn). Diets were balanced for all other nutrients. A fifth, synthetic (SYN), diet was formulated based on the AIN-93G diet. Each diet was fed to five weanling rats for six weeks.

There was no difference in rat growth between the five diets, but zinc retention (zinc intake minus excretion) was lower for rats fed the CONV diet compared with the other diets. Zinc concentrations in blood plasma, muscle and liver were similar for the grain-based diets but zinc concentration in the femurs was less for the rats fed the CONV diet.

These results show that marginal zinc deficiency may have been induced by the CONV diet with significantly lower risk of deficiency under the ORG diet. Zinc deficiency amongst humans is potentially widespread. Thus the claim could be made that organically grown wheat has greater nutritional value in terms of zinc nutrition, particularly for individuals with sub-optimal zinc status.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to a copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Julie L. Kitchen

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Chapter 1

General Introduction

Organic farming has been defined as a production system which excludes the use of synthetically produced fertilisers, biocides, growth regulators and livestock feed additives such as antibiotics and growth hormones (USDA, 1980). Instead, practices such as biological pest control, mechanical cultivation and crop rotations are utilised alongside inputs such as legumes, green manures, animal manures, crop residues, compost and mineral bearing rocks (NASAA, 1993).

The organic foods industry has grown remarkably, both in Australia and overseas, during the past decade. In the UK the market for organically grown products increased 2.3 fold in the period 1991-1996, to a value of £180 million, around one percent of the total food market (Twyford-Jones and Doolan, 1998) and similar to that in other parts of Europe. In Australia the relative expenditure on organically grown foods is much less than in Europe. However, here too the trend is showing a steady increase from an average of 0.13 percent of total food expenditure in 1990 to 0.2 percent in 1995 (Hassall and Associates, 1996). The Organic Federation of Australia is currently implementing plans to promote the consumption of organically grown foods so that, by 2006, four percent of all foods consumed in Australia are certified organic (VCG, 2000).

The growth of the 'organic foods' market has, necessarily and understandably, been reflected in an increase in the production of organically grown food. For example, Australian organic production has increased from 0.3 percent of the total agricultural area in 1990 to 0.8 percent in 1995, with projections of 1.9 percent by 2005 (Hassall and Associates, 1996) and four percent of total agricultural production by 2006 (VCG, 2000). Sweden has one of the highest proportions of total farmland farmed organically. The Swedish government has aimed to increase the area of organic production from 1.6 percent of the total farmland in 1996 to 10 percent by 2000 (Twyford-Jones and Doolan, 1998). Figures from 1999 (Kallander, 2000) indicate that Sweden was on track to meet this aim. Furthermore, 8.4 percent of total farmland is dedicated to organic production in both Austria (Pohl, 2000) and Switzerland (Niggli, 2000).

This growth in the production of organically grown food has also been fuelled by the premium prices that consumers are prepared to pay for food that has been grown organically. Premiums are largely acceptable to consumers due to the fact that the ‘certified organic’ label has been promoted as representing food of high nutritional value and virtually free of harmful chemicals, that has been produced in a safe and ecologically sustainable manner (OPAC, 1992).

Ecological sustainability includes nutritional sustainability (maintenance of nutritional status of soil, plants and animals) within organic systems. Due to the low inputs in many organic systems and the export from farms of nutrients via produce, scientists have questioned the nutritional sustainability of organic systems (Dumaresq and Derrick, 1992; Conacher and Conacher, 1998; Entz *et al.*, 1998). Nutritional sustainability is reflected, in part, by the maintained capability of plants to adequately access the nutrients that are required for growth and development. The current study aims to assess the capability of operating organic systems to deliver nutrients required for plant growth and in this way to assess the nutritional sustainability of organic systems in comparison to neighbouring conventional systems.

Both proponents and opponents to the 'organic food' movement ask the questions; 'What is the difference between organically and conventionally grown food?' and 'Are the premiums paid for organically grown food justified on a nutritional basis?'. Because of the expansion of the organic foods industry and its increased acceptance, this question has become an important one. Indeed, are there measurable differences between foods produced under different farming systems and, if there are, what value are these differences to the consumer?

The current study attempts to answer these questions, at least in part, firstly by comparing aspects of the composition of grain grown under each system and secondly by investigating, in a rat model, the biological relevance of selected compositional differences.

Chapter 2

Literature Review

2.1 Introduction

This review summarises available literature covering past and present work relating to the topics of organic systems, nutritional composition and value of food, particularly wheat, and the nutritional value of organically and conventionally grown wheat. It begins by highlighting the importance of good and balanced nutrition within, firstly, the plant kingdom and secondly, the human population. From there, the focus moves to organic farming, raising the issue of the nutritional value of organically grown foods. The focus then shifts again, this time to the factors that can influence the nutritional composition of food, particularly wheat, and to bioavailability and the factors that influence the nutritional value of these foods to us as consumers.

2.2 The Importance of Nutrition

Nutrition is defined as the supplying or receiving of nourishment (The Australian Concise Oxford Dictionary, 1987). The importance of adequate nutrition is emphasised by the diseases and conditions that occur in both plants and animals, either directly or indirectly due to nutrient deficiencies. Nutrients are chemical substances that promote normal functioning of a plant or animal (Burk and Solomons, 1985) and include amino acids, minerals and vitamins.

2.2.1 Plant Nutrition

Nutrient deficiencies in plants are mostly due to low availability of that nutrient from the soil and can usually be corrected through the application of appropriate fertilisers. The old and weathered Australian soils are commonly nutrient deficient, particularly in phosphorus and trace elements, for example, manganese, copper and zinc.

Nitrogen and phosphorus deficiencies are most common amongst plant species due to the high requirements of plants for these nutrients. However, trace element deficiencies are also common and often growth limiting where nitrogen and phosphorus fertilisers are applied. This is because plant growth is increased due to the increased nitrogen and phosphorus supply, thereby increasing the requirement for trace elements, which may not be adequately supplied by the soil.

Symptoms of severe deficiency in plants include chlorosis and necrosis of the leaves. However, most nutrient deficiencies, even when marginal, result in a reduction of plant growth and a loss of production for the agriculturalist.

Nutrient deficiencies can also have secondary effects on plant health through interactions with disease. It has been shown that there is a significant negative correlation between the zinc status of wheat plants (determined by leaf analysis) and the severity of root rot caused by the fungus, *Rhizoctonia solani* (Thongbai *et al.*, 1993). Furthermore, the application of zinc to barley resulted in an asymptotic rate-wise reduction in the area of bare patch (a symptom of *Rhizoctonia* infection) (Thongbai *et al.*, 1993). The same interaction between zinc and *Rhizoctonia solani* was also shown to exist in medic (Streeter, 1998). The reduction in symptoms with the addition of zinc was shown to be due to an increased tolerance towards the pathogen because infection levels were not reduced (Streeter, 1998).

Another nutrient-disease interaction is that between manganese and the take-all fungus, *Gaeumannomyces graminis.* It has been shown that the severity of take-all is greater in plants that are manganese deficient (Wilhelm *et al.*, 1990).

The form of the nutrient applied as a fertiliser can also affect the disease status of a plant. Wheat fertilised with ammonium, compared to nitrate, as the nitrogen fertiliser was found to have fewer take-all symptoms (Huber, 1981).

The possibility of nutrient deficiency in plants, however, has more far reaching consequences than reduced agricultural production. Nutrient deficiency in plants usually means that plant parts contain only low levels of that nutrient. These very plant parts comprise a major proportion of the human diet in most areas of the world. In this way, nutrient deficiency within plants can lead to nutrient deficiency in animals and humans, especially in those who practise subsistence agriculture or who consume only locally grown food.

2.2.2 Human Nutrition

Possibly one of the most well known nutrient deficiencies in humans is iron deficiency which causes anaemia. Anaemia is present in almost all socio-economic groups around the globe but is especially common amongst infants and women, particularly those of reproductive age and adolescents (Nelson, 1996; Walters, 1974). Anaemia has a higher occurrence in developing countries and in low socio-economic groups generally but prevalence can be reduced as dietary iron intake is increased due to improved provision of iron rich foods and increased awareness of the disorder (Walters, 1974).

Another well known nutritional disease is osteoporosis, which is caused by a calcium deficiency. However, it is less well known that deficiencies in the trace elements copper (Danks, 1980) and possibly zinc and manganese (New *et al.*, 1997; Strause *et al.*, 1994) have also been implicated, by some researchers, in the onset of osteoporosis, possibly due to their role as cofactors of enzymes involved in the deposition of calcium in bones (Saltman and Strause, 1993). Phosphorus deficiency can also lead to reduced bone mineralisation due to the high requirement of phosphorus within the skeleton. Bone ash contains approximately half as much phosphorus as calcium (McDonald *et al.*, 1988)

Recently there has been a link established between selenium deficiency and certain cancers. Selenium, an essential trace element involved in cell proliferation, has been shown to stimulate cancer cell growth at low levels but, as an antioxidant, is inhibitory to cancer at higher levels (Nano *et al.*, 1989). Clark *et al.* (1996) have shown that in areas with low levels of selenium in the soil (and hence low levels in locally produced food), selenium supplements significantly reduced the incidence of lung, colorectal and prostate cancers, although not reducing skin cancer incidence. Blot *et al.* (1995) also reported that supplementation with selenium plus carotenoid derivatives, also anti-oxidants, reduced deaths due to cancer in a part of China with a high incidence of oesophageal and stomach cancer.

Amino acids, the components of protein, are also important in nutrition because they are required for the synthesis of protein, used in growth and repair of tissues and in hormone production and other metabolic processes. The production of hormones can be blocked by deficiencies in specific amino acids. An example is adrenalin which is derived from the amino acids tyrosine and phenylalanine (Gurin and Delluva, 1947). Arginine, another amino acid, is critical in the production of sperm (Albanese, 1950).

Amino acid imbalances have also been connected to Chronic Fatigue Syndrome (CFS) with sufferers having unusually high levels of tyrosine and low levels of alanine, glutamic acid (McGregor *et al.*, 1996) and serine (I. Buttfield, Specialist Physician, *pers comm.*) compared with healthy individuals.

Results from laboratory experiments have also suggested that Acquired Immunodeficiency Syndrome (AIDS) may be caused by a deficiency in the amino acid, cysteine, induced by a virus (Droge *et al.*, 1988). Clinical studies have shown that levels of cystine, a derivative of cysteine, are correlated with lymphocyte activity in both people suffering from AIDS and in healthy people (Eck *et al.*, 1992). This indicates that cysteine is possibly involved with immune function.

Many of the human conditions discussed here are not caused by nutritional deficiency alone but in all of these it is a contributing factor. All of these essential nutrients are sourced through the food we consume. The large variety of foods available, particularly in western society, should mean that the nutrient status of the population is not affected by a lower level of these nutrients in one food type (eg. grain) because nutrients can be sourced from other foods. However, if this is the case, why does it seem that there is an increasing incidence of diet-related disorders within western society today?

The nutritional value of any food can be defined in terms of its efficiency to supply nourishment. This encompasses more than just the composition of the food in terms of essential nutrients and calories, extending also to the bioavailability of the components.

Many people who consume food labelled as 'organic' believe that this food is more adequately supplying them with nutrients than food not labelled 'organic' (Maga, 1983). Although this view has not been substantiated, it is used to promote organically produced food (Figure 2.1).

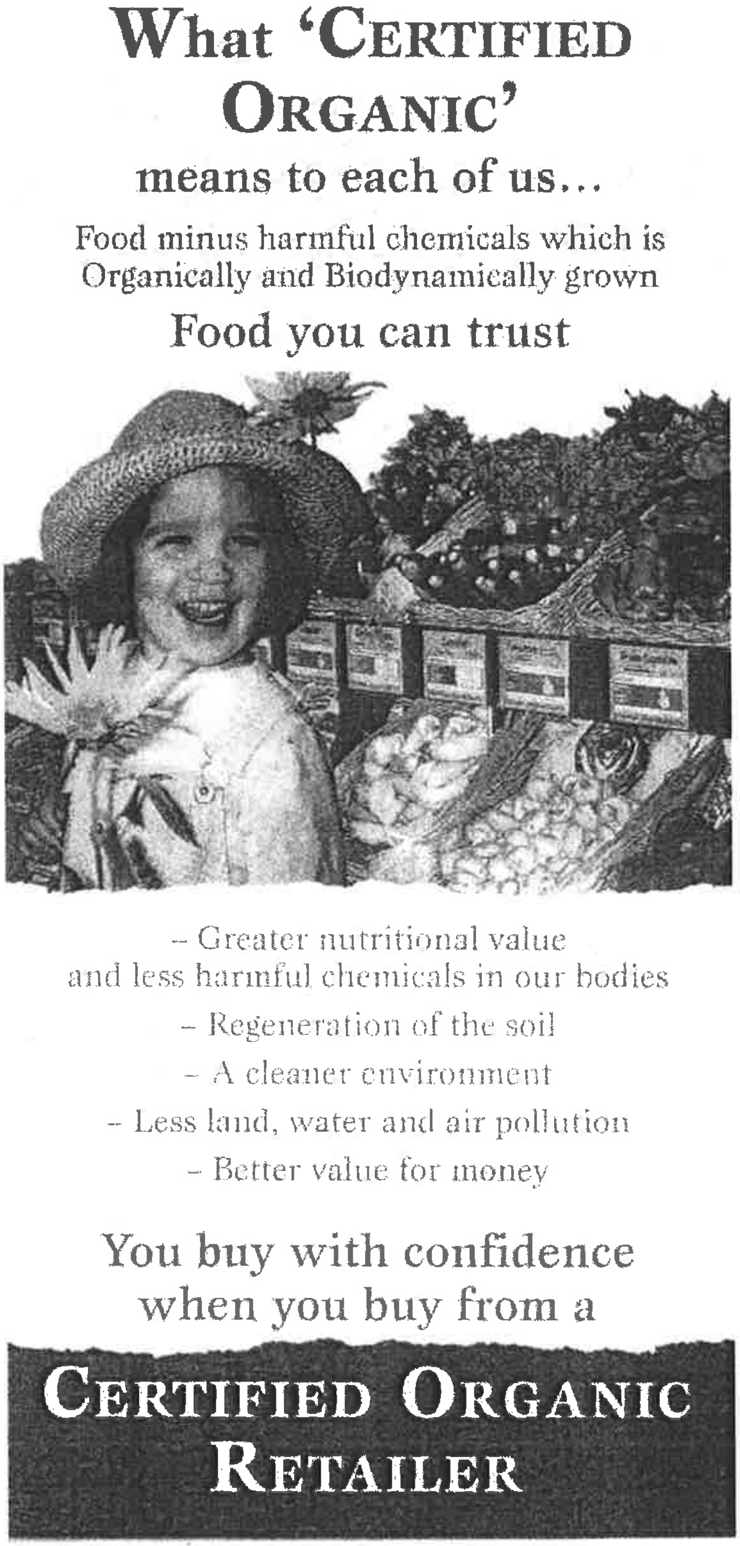
This literature review aims firstly to determine what is meant by the 'organic' label and how it differs from unlabelled, 'conventional', produce in terms of production and secondly, to review the scientific basis for the view that methods of production can alter the nutritional value of food.

2.3 Organic Farming

2.3.1 Introduction

Most Australian rain-fed agro-ecosystems involve extensive manipulation of the natural ecosystem by farmers, including tillage, monoculture, the use of agrochemicals and the addition of synthetic fertiliser. Tillage and monoculture have been used in agro-ecosystems since Australia was opened up for farming but synthetic inputs, including fertilisers and pesticides, have increased over time. In particular, the Green Revolution of the late 1960s resulted in increased synthetic inputs as part of a successful attempt to increase agricultural production to feed the growing world population (Brown, 1970).

However, as a consequence of natural ecosystem manipulation to achieve high production and maximum profit, ecosystems have deteriorated. As part of this deterioration, many Australian agricultural areas under regular cultivation have undergone rapid soil degradation, reflected in soil structure decline, increased salinisation, acidification and soil erosion and reduced organic matter content (Conacher and Conacher, 1982).



What 'CERTIFIED ORGANIC'

means to each of us...

Food minus harmful chemicals which is
Organically and Biodynamically grown

Food you can trust

– Greater nutritional value
and less harmful chemicals in our bodies
– Regeneration of the soil
– A cleaner environment
– Less land, water and air pollution
– Better value for money

You buy with confidence
when you buy from a

CERTIFIED ORGANIC RETAILER

Figure 2.1 A brochure produced by the Organic Retailers and Growers Association of Australia (ORGAA) advertising the advantages of buying and consuming certified organically grown food, including 'greater nutritional value'.

Many natural processes contributing to nutrient cycling can also be altered through the addition of synthetic inputs. Examples of such cycles are the phosphorus, sulphur, nitrogen and organic carbon cycles which can be unbalanced or accelerated by the addition of substrate in the form of synthetic nutrients (Adedeji, 1986) and changed when biocides alter microbial populations (Taiwo and Oso, 1997) which play an important role in releasing these nutrients from organic matter. Synthetic nutrients are those present in fertilisers that have been synthetically compounded (NASAA, 1993) and are usually water soluble to enhance uptake by plants.

During the early 1980s, soil degradation was recognised as a threat to maintaining high production, and sustainability of production became a widespread concern (Quick, 1985). Conservation farming, high input farming and organic farming have all been developed, or further developed, as a response to the sustainability crisis, each with a different emphasis (Quick, 1985; Prasad and Power, 1997).

2.3.2 Definitions

Organic farming, also called biological or ecological farming, is difficult to define and management techniques vary greatly among farmers. However, it is defined by the National Association of Sustainable Agriculture, Australia (NASAA) as a form of sustainable agriculture:

> 'A system of agriculture able to balance productivity with low vulnerability to problems such as pest infestation and environmental degradation, while maintaining the quality of the land for future generations.
>
> 'In practice this involves a system which avoids or largely excludes the use of synthetically compounded fertilisers, pesticides, growth regulators, livestock feed additives and other harmful or potentially harmful substances. It includes the use of technologies such as crop rotations, mechanical cultivation and biological pest control; and such materials as legumes, crop residues, animal manures, green manures, compost, other organic wastes and mineral bearing rocks. The intention is to encourage natural biological systems.' (NASAA, 1993, p. 1)

Conversely, 'conventional' agricultural systems today rely heavily on biocides for pest and weed control and synthetic fertilisers to provide crop nutrition. Conventional farming systems in Australia generally use less biocides and synthetic fertilisers than those in USA and Europe (Burlace, 1990). However, these products are still greatly relied upon in Australian farming today (FAO, 1990-1998a).

The Organic Produce Advisory Committee (OPAC) is somewhat more descriptive when they define 'organic' as:

> '...produced in soils of enhanced biological activity, determined by the humus level, crumb structure and feeder root development, such that plants are fed through the soil ecosystem and not primarily through the soluble fertilisers added to the soil. Plants grown in such systems take up essential soluble salts that are released slowly from humus colloids, at a rate governed by warmth. In this system, the metabolism of the plant and its ability to assimilate nutrients is not overstressed by excessive uptake of soluble salts in the soil water (such as nitrates). Organic farming systems rely to the maximum extent feasible upon crop rotations, crop residues, animal manures, legumes, green manures, mechanical cultivation, approved mineral-bearing rocks and aspects of biological pest control to maintain soil productivity and tilth, to supply plant nutrients and to control insects, weeds and other pests.' (OPAC, 1992, p. 3)

The term 'organic' does not necessarily refer to the inputs of the system being organic in nature (ie. plant or animal based), but rather to the concept that the farm is like an organism, made up of interactive components: minerals in the soil, organic matter, microorganisms, plants, insects, animals and humans (Lampkin, 1994a). Management techniques focused on one of these components will likely also affect other components. Organic farming systems aim to take these secondary effects of management into account so as to minimise the disturbance to the natural ecosystem.

Contrary to common belief, organic farming is not a reversion to the farming practices of the nineteenth century. Although some 'traditional' methods may be used, organic farming incorporates these with conservation farming practices, modern technology and current scientific knowledge and recommendations (USDA, 1980; Reganold, 1989).

Organic farming practices in Australia vary in certain aspects from those found in Europe, although both still fit into the definitions of organic farming as given here. Farming in Australia is generally less intensive than farming in Europe, mainly due to the low natural fertility of the soils in Australia and the harsh climatic conditions. For this reason, organic farming in Europe is much more easily able to incorporate practices such as the use of composts and animal manures as a nutrient source and the manipulation of grazing for weed control. The implication of these differences (particularly the former) is that the organic farming research based in Europe may not always be applicable to organic farming in Australia because of quite different nutrient sources.

2.3.3 Advantages and Disadvantages of Organic Farming

Like any other enterprise, organic farming has many advantages and disadvantages. In this case, most are with respect to economics, sustainability and adoption

The first disadvantage of organic farming, frequently highlighted by opponents, is that yields are often low. In the scientific community there are mixed reports on the yield results of organic farming but it has been reported to be lower than comparable conventionally grown wheat in most cases. Halberg and Kristensen (1997) reported that on Danish mixed farms, grain yields of organic farms are consistently lower than conventional farms. Oberson *et al.* (1993) obtained the same result for winter wheat in Switzerland. Lockeretz *et al* (1981) reported from the USA that organically grown wheat yielded 43 percent lower than conventionally grown wheat over two years. An on-farm comparison in New South Wales reported organic yields that ranged from 18 to 84 percent lower than conventional yields (Derrick, 1996). These reports seem extreme compared with reports from Canada (Entz *et al.*, 1998), Western Australia (Deria *et al.*, 1996) and New South Wales (Dumaresq, 1992) of organically grown wheat yielding 28, 15 and 25-30 percent lower respectively than a regional average (or paired conventional paddocks in the Western Australian example). Stanhill (1990) reported yield loss due to organic farming of wheat ranging from 10 to 20 percent and an average of 10 percent over crops generally, in a review of experimental comparisons of organic and conventional systems. However, he found when experimental results of organic systems were compared to district averages (ie. less experimental control), there were more instances where organic systems yielded exceptionally well, but also exceptionally badly, in comparison. Over a period of 14 years, wheat from the organic system in the English 'Haughley' experiment yielded 3 to 57 percent lower than the county average in that year and there was one year (1957) in which the organic system yielded more than the county average by 37 percent (Stanhill, 1990). The organic wheat farms in the Victorian Wimmera also yielded more than the regional average by nearly 30 percent in 1989 (Dumaresq, 1992). This indicates that although organically grown wheat usually yields less than conventionally grown wheat, there are some examples of higher yields in organic systems. Reganold (1989) reported that the organic farm he studied in the USA produced comparable wheat yields to neighbouring conventional farms. Wynen (1994) reported that in south eastern Australia in the 1985/86 season, there were no significant differences in wheat yields between organic and comparable conventional farms. These data were not experimental data but actual yields reported by farmers. Stanhill (1990) suggested that commercial yields can be higher than yields in large experimental plots because commercial practices are readily modified with experience whereas experimental treatments are generally more strictly controlled.

In reality, organic farmers often expect and are satisfied with a lower yield than their conventional counterparts but there appears to be an effect of rainfall on the relative yield of organically compared with conventionally grown crops. In dry years farmers have observed that organically grown crops last longer than conventionally grown crops before they become drought affected (Wynen, 1994). This leads to the possibility of similar yields for organic farmers compared to conventional farmers in dry years. However, in wet years organically grown crops are more likely to be disadvantaged, unable to respond in the same way as conventionally grown crops to increased water supply, and hence yield far less. In such conditions, organically grown crops are probably limited by nutrient availability (Wynen, 1994; Halberg and Kristensen, 1997). This trend was also present in yield data reported by Dann *et al.* (1996) from experiments comparing an organic and a conventional farm over two seasons which varied widely in annual rainfall. This also correlates with yield results in the wetter European climate where organically grown wheat has yielded significantly lower than conventionally grown wheat (Oberson *et al.*, 1993; Halberg and Kristensen, 1997). In Denmark, regressions of potential yield and available water (including irrigation) in both organically and conventionally grown crops suggest that, under improved water supply, conventionally grown crops have a larger yield increase (Halberg and Kristensen, 1997).

Another reason for lower yields of organically grown crops is that often organic farmers in Australia sow their crops later than their conventional counterparts, using cultivation before sowing to control weeds. The potential yield of any crop, regardless of the system in which it is grown, is reduced when the growing season is shortened by late sowing (French and Schultz, 1984a), except in special cases, for example frost damage, where an early sown crop could be more susceptible due to its stage of development.

Furthermore, organic farmers do not always grow the highest yielding varieties of grain crops. This is because different end uses may be targeted by conventional and organic farmers, for example, animal feed compared with bread-making for human consumption (Halberg and Kristensen, 1997). Often organic farmers will have a direct market to millers and good bread-making varieties are requested rather than high yielding varieties.

Lower yields, obtained in many cases by organic farmers, have raised the argument from critics that, because of world population growth, organic farming is an inadequate method to feed the population. However, it is conceivable that less food may be required if the food produced was of higher nutritional quality, that is, containing higher bioavailable levels of limiting nutrients and amino acids and lower levels of anti-nutrients and toxins. In support of this hypothesis are the findings of Zhu and Haas

(1998) of increased energy expenditure by iron-depleted, but non-anaemic, women, compared to iron supplemented women, in a simulated15 km cycling time trial. Hence, food with a higher iron content would result in more efficient use of the energy provided.

Another disadvantage associated with the production of organically grown food in Australia is that farmers must often develop their own market (Wynen, 1994), often directly to processors or retail outlets. Currently, there are no bulk marketing organisations operating in Australia for organically grown produce. However, the marketing of organically grown produce has been developed further in Europe, where in several countries government assistance for conversion to organic farming has necessitated improved market structure (Tate, 1994).

An advantage of farming organically is that the economic viability of organic farming has been enhanced by higher prices that can be commanded for organically grown food. These premiums are an advantage associated with organic farming in Australia (Wynen, 1994). However, in countries (for example, Denmark) where government assistance has recently increased the number of organic producers, premium prices for food produced organically have in some cases been lowered or removed due to an oversupply of the niche organic market (Dubgaard and Holst, 1994).

Some consumers argue that the price premiums asked for organically produced food are unrealistic and will hamper the growth of the 'organic food' industry. However, T. Marshall (former NASAA board member, *pers comm.*) argued that conventionally grown food is indirectly subsidised by government agencies that deal with the environmental and health issues that are linked to the high use of fertilisers and biocides in agricultural production. It is possible, therefore, that the premium price asked for organically produced food is closer to the true value of the food.

Despite these problems, farmers continue to carry on organic farming. Their main stated reasons for this commitment are to maintain the health of their families and to reduce environmental degradation (Conacher and Conacher, 1982).

2.3.4 Organic Farming Systems Research

There are two main types of research in the area of organic farming; research in which organic farming systems are compared to either conventional systems, or to other organic systems (Lampkin, 1994b). When organic farming systems are compared with other organic systems, the aim is to improve organic farming techniques and effectiveness and therefore, has direct benefit to producers. The focus of the current study, however, is on the comparison between organic and conventional farming systems. This is essentially systems research and is important in producing information required in policy decisions and also information for conventional farmers who may be considering conversion to organic farming and desire experimental evidence of the outcomes (Lampkin, 1994b).

Most experimental research is reductionist, undertaken in the form of replicated factorial experiments. This means that replicated experiments are set up in which all variables are maintained as constant except for those of specific focus, which are adjusted and monitored. When comparing agricultural production systems, in which there are simultaneous interactions occurring between a number of environmental and management variables, it is not feasible to use a reductionist approach because the system must be investigated as a whole. If individual variables within the system are restricted in this type of experiment, the performance of the system could easily be altered (Lampkin, 1994b). For this reason, it is advised in systems research (Shennan *et al.*, 1991) that the whole system be initially examined and documented and only then that specific relationships within the system, as are identified by the initial documentation, be targeted by reductionist experiments.

There are two contrasting methods of system comparison. The first of these is a long-term replicated experiment comparing different systems in totality and the second is an on-farm comparison of systems (Shennan *et al.*, 1991). These are complementary research methods, each having its own advantages and disadvantages.

Replicated Systems Experiments

A replicated systems experiment of limited replication was carried out comparing organic production with conventional and other biological production systems at Roseworthy, South Australia, by Penfold *et al.* (1995). The advantages associated with this method (Shennan *et al.*, 1991) are that confounding variables such as soil type and climate are removed and there is true replication of the treatments. Another advantage is that the researcher has full control over management decisions, although in the study by Penfold *et al.* (1995), practising farmers in each system were involved in the decision making for the treatment of that system.

A disadvantage of replication experiments comparing systems is that the experiment is conducted in a single location and often using only one set of management practices so that the results obtained may not be relevant to other geographical areas or varying managerial techniques (Shennan *et al.*, 1991). They state that another disadvantage is that where replicated experiments are used, there is usually a trade off between the size of the plots and the amount of replication and, therefore, the degree to which the results are representative of that system.

Converting a conventional farming system to an organic system can take a number of years and therefore the first years of any replicated systems experiment will be describing the transition phase and not the final organic system (Shennan *et al.*, 1991). Because of the short term research funding that is common in Australia today, it is therefore difficult to undertake replicated experiments comparing true organic and conventional systems.

On-Farm Comparisons

The alternative to replicated experiments for systems research is on-farm comparisons. In the context of organic farming systems, this means that operating organic farms are compared with neighbouring conventional farms. This would ideally involve across-the-fence comparisons so that environmental variation is minimised.

A comparison of this sort, between neighbouring organic and conventional farms was carried out at Ardlethan, New South Wales, by Dann *et al.* (1996). However, this research focused mainly on a single pair of farms. Because on-farm comparisons usually involve a number of pairs of contrasting farms and therefore various sites and management practices within the same system, this type of research has the benefit of testing the robustness of any relationships identified. On-farm comparisons have the advantage that the management techniques used within the system are realistic because they represent decisions made by actual farmers (Shennan *et al.*, 1991). Other advantages include the fact that the transitional phase can be avoided when on-farm comparisons are used rather than replicated experiments and also that the researchers involved interact directly with the farmers, resulting in valuable information exchange.

Research involving on-farm comparisons, however, is disadvantaged because potentially confounding variables such as climate, soil type, topography, farm layout, financial circumstances of the farmer, management expertise and decisions with respect to planting date and soil preparation, must be accounted for (Shennan *et al.*, 1991; Lampkin, 1994b). This could mean more extensive data collection so that misinterpretation of analyses is avoided. Another disadvantage is that on-farm comparisons often involve pseudo-replication because of the impossibility of randomising the system treatment. The drawback to the use of pseudo-replication is that the effects attributed to system are assumed to be treatment effects, but could also be related to the confounding variables mentioned above, particularly if care is not taken in site selection. Other disadvantages of on-farm comparisons (Shennan *et al.*, 1991) include the extra time that is required to coordinate operations such as planting and harvesting and also that the researcher is not in complete control, leaving the management decisions to the farmer.

2.3.5 Quality of Organically Grown Food

Quality characteristics of plant products are much more difficult to define and quantify than yield characteristics and are dependent on the intended use of that plant product (Mengel and Kirkby, 1987). A good example of this is the low protein requirement for malting barley compared with the high protein requirement for feed barley.

Advocates of organic food have a strong belief that organically grown food is of higher quality than conventionally grown food. They claim that organically grown food tastes better, is nutritionally superior and is safer than conventionally produced food because it does not contain chemical residues (Maga, 1983), all aspects of quality.

In reality, organically grown food can not be guaranteed free of chemical residues because it is not possible to exclude all sources of chemical residue, including dust particles, water courses and the food chain (for example, a rabbit consuming chemically contaminated grass dying within an organic system) (Burlace, 1990). This was illustrated by Di-Mare *et al.* (1995) in a survey comparing fruit and vegetables grown using biological/biodynamic (essentially organic) and traditional (conventional) methods. Chemical residues were found in 14.8% of the organically grown vegetables and 25% of the organically grown fruit. However, this was significantly lower in each case than the conventionally grown vegetables and fruit (50.0% and 82.2% respectively, contained chemical residues). Their study does not report the actual levels of chemical residue detected in the food, which could have varied greatly depending on the production system.

Maga (1983) believes that consumers of organically grown food confuse food safety with nutrition. He infers that consumers could often be referring to the fact that organically grown produce contains fewer chemical residues when they claim that the food is more nutritious. Leverton (1973) disputes the possibility of organically grown foods having different nutritional value to conventionally grown foods.

Research comparing the nutritional composition of food produced in organic and conventional systems has produced a myriad of results. These probably reflect a variety of environments, genotypes and cultural techniques used in each of the organic and conventional systems (refer to section 2.4). Comparison research of this type can be divided into two types. The first is the survey, where food samples have been obtained, either from retailers or growers, and analysed. The second type of comparison research is the controlled experiment, where food is grown within defined treatments and the products analysed.

Survey type research is limited in many cases due to a lack of knowledge regarding the treatments applied within each system. Particularly when undertaking a 'shopping basket' survey where samples are obtained through retailers, there is no control over obtaining comparable organically and conventionally grown produce. Another disadvantage associated with such a survey is that in many countries (currently including Australia) legislation does not prevent people from marketing conventionally grown produce as organically grown (T. Marshall, former NASAA board member, *pers comm.*). Certified organic growers will, however, label their produce with their certification number to guarantee its authenticity.

Survey type studies are summarised in Table 2.1. Surveys investigating vegetables include a rigorous survey by Leclerc *et al.* (1991) in which concentrations of calcium, magnesium, potassium, nitrogen, phosphorus, copper, zinc, manganese, iron and nitrate were analysed in carrot and celeriac. The organically grown celeriac was found to contain less zinc, nitrogen and nitrate and more phosphorus than that conventionally grown. There were no significant differences found between organically and conventionally grown carrots. A survey by Pommer and Lepschy (1985), however, found carrots to be lower in calcium, potassium and nitrate and higher in sodium and copper (no difference in magnesium or zinc) when they were organically grown. Knight (1990) reported higher magnesium contents of certain vegetable crops sold as 'organic' compared to those sold without the 'organic' label. O'Reilly (1993) surveyed organically and conventionally grown cabbage and found lower sodium, potassium, calcium and magnesium and greater iron and cobalt when the cabbage was organically grown. Smith (1993) reported much higher concentrations of most minerals (including many uncommon trace minerals) in apples, pears and potatoes that were marketed as organically grown compared to those purchased as conventional produce.

Smith (1993) found a similar result to that of the fruit and potatoes when he compared wheat and sweet corn that were marketed as organically grown with those that were conventionally produced. The organically grown wheat was greater in calcium, copper, magnesium, manganese, phosphorus, potassium, sulphur and zinc and lower in iron and sodium compared to conventionally grown wheat (Smith, 1993). When Pommer and Lepschy (1985) compared organically and conventionally grown wheat, they found no difference except that there was a trend towards the organically grown wheat having greater copper levels (in agreement with Smith (1993)). O'Reilly (1993) also investigated wheat, finding lower potassium and higher magnesium when it was produced organically. However, his results were somewhat confounded by wheat variety and the use of an organic system in conversion.

Table 2.1 Summary of the results of survey type comparisons of the composition of organically and conventionally grown produce.

Element	Org > Conv	No Difference	Conv > Org
B	Apple, Pear, Potato, Corn (Sm)	Wheat (Sm)	
Ca	Apple, Pear, Potato, Corn, Wheat (Sm)	Carrot, Celeriac (Lec) Wheat (P & L)	Carrot (P & L) Cabbage (O'R)
Co	Cabbage (O'R) Apple, Pear, Corn (Sm)		Potato, Wheat (Sm)
Cu	Carrot, Wheat (P & L) Potato, Corn, Wheat (Sm)	Carrot, Celeriac (Lec) Apple, Pear (Sm)	
Fe	Cabbage (O'R) Apple, Pear, Corn (Sm)	Carrot, Celeriac (Lec)	Potato, Wheat (Sm)
K	Pear, Potato, Corn, Wheat (Sm)	Carrot, Celeriac (Lec) Apple (Sm) Wheat (P & L)	Carrot (P & L) Cabbage, Wheat (O'R)
Mg	'Certain vegetables' (Kn) Apple, Pear, Potato, Corn, Wheat (Sm) Wheat (O'R)	Carrot, Celeriac (Lec) Carrot, Wheat (P & L)	Cabbage (O'R)
Mn	Apple, Pear, Corn, Wheat (Sm)	Carrot, Celeriac (Lec) Potato (Sm)	
N		Carrot (Lec)	Celeriac (Lec)
Na	Carrot (P & L) Apple, Pear, Potato, Corn (Sm)	Wheat (P & L)	Cabbage (O'R) Wheat (Sm)
P	Celeriac (Lec) Apple, Pear, Corn, Wheat (Sm)	Carrot (Lec) Potato (Sm)	
S	Potato, Corn, Wheat (Sm)		Apple, Pear (Sm)
Zn	Pear, Potato, Corn, Wheat (Sm)	Carrot (Lec) Carrot, Wheat (P & L) Apple (Sm)	Celeriac (Lec)

Org (organically grown); Conv (conventionally grown); Sm (Smith, 1993); Lec (Leclerc *et al.*, 1991); P & L (Pommer and Lepschy, 1985); O'R (O'Reilly, 1993); Kn (Knight, 1990).

There have also been many comparisons of the nutrient content of organically and conventionally grown food from within controlled experiments. These are generally more useful than surveys due to the use of defined treatments. Results are summarised in Table 2.2. Warman and Havard (1997) compared the nitrogen, potassium, phosphorus, sodium, calcium, magnesium, sulphur, boron, iron, manganese, copper and zinc levels in organically and conventionally grown carrots and cabbage. Apart from a higher sulphur content in carrots that were organically grown, there was either very little difference, or differences that were inconsistent over the three seasons, between the produce from organic and conventional systems. Boeringa and de Vries (1989) reported that carrots grown on their experimental organic farm had greater potassium and less nitrate than the carrots grown on their 'current farming practices' farm. Jorhem and Slanina (2000) measured the zinc content of potatoes and found no difference between those grown on organic and conventional farms.

The zinc and chromium contents of organically grown wheat and rye were compared to those conventionally grown and found not to differ, in the study by Jorhem and Slanina (2000). Boeringa and de Vries (1989) also grew wheat on their experimental farms and found that the wheat from the organic farm contained less iron than that from the 'current practices' farm. Belderok (1978), in his analysis of wholemeal wheat flour found greater levels of potassium and sodium when it was organically produced, compared to conventionally produced. Higher phosphorus levels were reported in wheat grown conventionally in New South Wales than in organically grown wheat (Dann *et al.*, 1996; Derrick, 1996). This was shown to be due to the phosphorus fertiliser applied in each system because the grain phosphorus levels were dramatically increased when superphosphate was added to the organic system and reduced when phosphate rock was added to the conventional system (Dann *et al.*, 1996). Derrick (1996) also reported that the grain of the organically grown wheat had higher concentrations of chlorine, zinc and copper compared to conventionally grown wheat on the neighbouring farm and suggested that these differences should really be compared in a feeding trial to determine the true nutritional quality of organically and conventionally grown wheat. He also found a non-significant trend towards lower manganese concentrations in the organically grown grain.

Table 2.2 Summary of the results of controlled experiment comparisons of the composition of organically and conventionally grown produce.

Element	Org > Conv	No Consistent Difference	Conv > Org
B		Carrot, Cabbage (W & H)	
Ca		Carrot, Cabbage (W & H)	
Cl	Wheat (Der)		
Cr		Wheat, Rye (J & S)	
Cu	Wheat (Der)	Carrot, Cabbage (W & H)	
Fe		Carrot, Cabbage (W & H)	Wheat (B & dV)
K	Carrot (B & dV) Wheat flour (Bel)	Carrot, Cabbage (W & H)	
Mg		Carrot, Cabbage (W & H)	
Mn		Carrot, Cabbage (W & H)	Wheat (Der)
N		Carrot, Cabbage (W & H)	
Na	Wheat flour (Bel)	Carrot, Cabbage (W & H)	
P		Carrot, Cabbage (W & H)	Wheat (Der)
S	Carrot (W & H)	Cabbage (W & H)	
Zn	Wheat (Der)	Carrot, Cabbage (W & H)	

Org (organically grown); Conv (conventionally grown); W & H (Warman and Harvard, 1997); Der (Derrick, 1996); J & S (Jorhem and Slanina, 2000); B & dV (Boeringa and de Vries, 1989); Bel (Belderok, 1978).

In a review by Woese *et al.* (1997) comparing organically and conventionally grown foods, mostly based on European research, the protein concentration of wheat and other cereals was reported to be lower in organic systems. This is consistent with results obtained in Canada (M. Entz, The University of Manitoba, *pers comm.*) and Sweden (Haglund *et al.*, 1998). However, with rye it was shown that the baking quality of the grain was no different between the organic and conventional systems, despite a lower protein concentration of the grain produced in the organic system (Bolling *et al.*, 1986, cited by Woese *et al.*, 1997). This seems to implicate differences in the amino acid composition of the rye protein. It was found for wheat, however, that bread baked using wholemeal flour from organic farming systems was drier, less elastic and had a smaller loaf volume than bread baked using wholemeal flour from a conventional farming system (Haglund *et al.*, 1998). They suggested that an alternative recipe may improve some of the baking properties of the wholemeal flour from organic farming systems.

All of these quality assessments are based on the chemical composition of food. Vogtmann (1990) introduced entirely new concepts when he suggested that although these parameters may be important, they do not take account of the vitality and structural energy of food. He and his colleagues are developing novel methods for assessing these new parameters including copper chloride crystallisation (mixing plant extract with copper chloride solution and comparing crystallisation patterns) and measuring low level luminescence (transmitted by all living cells). Popp (1988, cited by Vogtmann, 1990) has identified samples of organically and conventionally grown food with 90-98 percent success using low-level luminescence. He claims that higher quality food transmits more photons at the same level of caloric energy and food produced in organic systems consistently transmits more photons than food from conventional systems. However, these methods are yet to be related to the nutritional value of the food and are not widely accepted by the scientific community.

Another unusual quality parameter, but one that has been deemed valid (Plochberger and Velmirov, 1992), is the food preference of animals given a selection of diets. Here, the animals may select one diet over another due to taste, texture, aroma or nutrient requirements. Plochberger (1989) provided hens with a selection of either organic or conventionally grown beets, after initially feeding them on one or the other. She found that initially the hens consumed more of the diet that they were used to but as the experiment progressed, both groups of hens consumed more of the organically grown beets. Although no reason is given as to why the hens selected the organically grown beets over those conventionally grown, this experiment indicates that animals can possibly discriminate between organic and conventional feeds. Unlike humans, animals will not be influenced in their selection by advertising campaigns or environmental concerns and therefore, their discrimination must be due to something inherently different between the two feeds.

The effects on animals of either organic or conventional diets have been assessed by a number of researchers. Plochberger (1989) monitored weight gain of hens fed either organic or conventional rations and also measured the weight of eggs produced. The only differences in weight gain were found 4-8 weeks after hatching in the first generation, when conventionally fed hens had significantly greater weights. However, in the second generation of hens fed on the two diets, no differences in weight gain were observed until 32 weeks after hatching when the organically fed hens had greater weight than those fed conventionally. In both generations, the egg weights of organically fed hens were significantly greater then those fed conventionally. This effect was not due to shell weight but rather to the proportion of albumen and yolk within the egg. Eggs produced by hens fed the organic diet had a greater percentage of yolk and less of albumen (Plochberger, 1989). The viability of young rabbits (when parents were fed on either organic or conventional diets) was assessed by measuring number of dead and live young at birth and number of young surviving to 2 months (Maurer, 1990). It was found that for those rabbits fed organic diets, there were more young alive and less dead at birth and a greater number surviving to 2 months. McSheehy (1977) also assessed litter size, using mice that were fed diets formulated using wheat grown organically or conventionally. He found that there was no difference between the diets in litter size, weaning ability or in the time between successive litters.

In a pioneering study, McCarrison (1926) found that rats or pigeons fed basal diets supplemented with millet or wheat grown using cattle manure (organic amendment) were superior in growth compared to animals fed the basal diets supplemented with grain grown using chemical fertilisers (conventional amendment). The studies by McCarrison (1926) and McSheehy (1977), and many other similar studies, are often quoted in support of the nutritional superiority of organically grown foods. However, these studies did not compare organic and conventional systems as we know them here in Australia, but rather compared food grown using organic amendments (manures or compost) with food grown using inorganic or chemical fertilisers. Although organic amendments of this type are commonly used in European organic systems, they are not commonly used in Australian organic systems due to larger land areas and drier conditions and, therefore, studies such as these may not be relevant to the organically grown grain produced here in Australia.

Despite these alternative quality assessments done in Europe, the claims of nutritional superiority of organically grown food are as yet unsubstantiated, particularly for food grown in Australian conditions. However, regardless of the lack of convincing scientific data, a Rodale Press Survey showed that 62% of respondents in a mail survey believed organically grown food to have a higher nutritional value and no respondents believed organically grown food to be inferior to conventionally grown food (USDA, 1980). A more recent Chinese survey showed similar results: 64% of respondents stated nutrition (distinct from safety) as the factor influencing them to purchase organically grown food (Wang *et al.*, 1997)

Organically grown food is often marketed locally, even at roadside stalls in some parts of the world, and therefore can be picked in peak condition without significant deterioration. This may mean that food, particularly fruit and vegetables, purchased as organically grown is more nutritious but possibly due, not to the method of production, but to shelf-life (Maga, 1983). However, Vogtmann (1990) reported that cabbage fertilised with organic fertilisers had greater shelf-life, losing less dry matter during storage than cabbage grown with soluble mineral fertilisers. Storage losses were also less for other vegetables when they were organically grown (Knorr and Vogtmann, 1981 (cited by Knorr, 1982)). Wheat and other cereals that are grown organically are seldom sold to a local market and can often be stored for extended periods. Australian organic farmers may transport their grain hundreds of kilometres for processing and the processed product is, in some cases, exported (Twist, 1999).

2.4 Nutritional Composition of Wheat

2.4.1 Introduction

Nutritional composition of wheat is important because, along with rice, it is the plant product that supplies the greatest amount of calories and protein per capita in the world (FAO, 1990-1998b). In developed countries (averaged), the caloric and protein contribution per capita by wheat is much greater than rice (FAO, 1990-1998b) because much of the pasta, flour and bread are made from wheat. In developing countries (averaged) wheat and rice both contribute to the calorie and protein consumption (FAO, 1990-1998b). Especially in some developing countries, wheat is a staple cereal, the major dietary component.

Because of their widespread use, wheat and the other cereals contribute significantly to the recommended daily intakes (RDI) of important nutrients. These include nutrients such as thiamin (vitamin B1), niacin, riboflavin (vitamin B2), tocopherol (vitamin E precursor) and iron, calcium, magnesium and zinc (Simmonds, 1989) but also amino acids and other components. Cereal grain also provides energy and protein to meet the dietary requirements of the population (Simmonds, 1989).

Nutritional composition of wheat grain is determined partly by environmental conditions (including agro-ecosystem manipulation) and partly by genotype.

2.4.2 Minerals

Mineral contents of whole wheat grain determined by four different surveys are shown in Table 2.3. The large ranges shown reflect the effect of environment, seasonal and cultural, on grain mineral content. It appears that wheats grown in Australia tend to have lower grain phosphorus and potassium contents than those wheats grown in North America (Batten, 1994).

When wheat grain was milled and the bran separated from the endosperm (the white flour fraction), Peterson *et al.* (1983) showed that there is a greater concentration of minerals in the bran than in the flour. This result confirmed the results of O'Dell *et al.* (1972) showing the greater proportion of nutrients in the parts of the grain that become bran, compared to the endosperm. However, the mineral concentrations in the bran varied more between different locations than those in the flour (Peterson *et al.*, 1983). This indicates that the mineral concentrations in the bran are more susceptible to environmental or cultural differences which vary between location.

Table 2.3 Ranges in mineral contents of whole wheat grain (mg/kg) in four surveys.

	Ca	Mg	K	P	Zn	Mn	Fe	Cu
Batten, 1994 * (12)#	380-580	1370-1540	4110-4770	3160-4540	15.3-35.2	26.1-53.8	28.7-59.1	3.5-9.1
Davis *et al.*, 1984 * (124)	120-800	600-1900	2800-6400	3100-7600	20.0-73.0	16.0-77.0	25.0-137.0	1.0-12.0
Dikeman *et al.*, 1982 * (437)	409-710	1400-1730	4200-5820	3850-4700	22.9-31.8	42.0-63.2	32.7-54.0	4.3-5.8
Batten, 1994 ** (26)	300-580	970-1970	3840-5120	2680-4540	4.5-35.2	23.6-54.1	26.0-85.5	3.5-36.6
Davis *et al.*, 1984 ** (404)	80-800	200-2200	2800-7300	2500-9100	15.0-102.0	10.0-90.0	16.0-163.0	1.0-14.0
Batten, 1994 ** 'Aust.' (46)##	240-500	810-1570	3070-4650	1990-3940	7.0-37.2	22.7-60.0	23.6-69.4	1.9-14.2
Murphy and Law, 1974 ** (88)##			2600-4300		14.0-46.0	25.5-99.0	29.0-110.0	4.0-8.6

*Hard red winter wheat. **All wheat classes. #The sample size is indicated by the number in parentheses. ##Australian comparisons.

Environmental effects on mineral composition

Any environmental factor that causes a change in yield could affect the nutritional composition of the wheat grain. This is usually in an inverse relationship. For example, increased yield will often result in lower mineral concentration due to growth dilution and *vice versa* (Jarrell and Beverly, 1981). Dilution occurs because the same quantity of a mineral has been taken up by the plant but is divided among more grains when the yield is higher, hence there is a lower concentration of a particular mineral in any single grain. This can be referred to as a concentration or dilution effect. Environmental conditions, in this context, may include soil fertility (determined by soil type, soil pH, fertiliser application etc.), climate, micro-organisms, disease and cultural methods which affect competition for or acquisition of nutrients.

Time of sowing is a factor that can affect the mineral composition of the grain. Batten *et al.* (1999) reported that wheat sown earlier in the season had a greater phosphorus content in the grain. This may be due to a longer growing season providing greater opportunity for phosphorus uptake.

Green manuring is one cultural method reported to change the composition of wheat grain. Although statistical analyses are not presented, green manuring is reported to have increased the grain phosphorus composition of wheat in all tested combinations of green manure and fertiliser except when ammonium sulphate was applied with the green manure (Bains, 1949).

The most basic cultural method which will affect grain mineral content is the addition, as fertiliser, of that mineral. For example, plants grown under low phosphorus conditions contained less phosphorus in the grain than those grown with adequate phosphorus (Batten, 1986; Batten and Wardlaw, 1987). Obviously, where there are more available minerals in the soil, the plant, in the absence of inhibition, will take up more of those minerals and, in most cases, this translates to a greater mineral content in the grain.

Gillman and Fox (1980) showed that after eight years of concentrated superphosphate application, the cation exchange capacities in the surface horizon of three different soils investigated had increased to a degree that was deemed agronomically significant. This reduced the leaching of applied calcium, magnesium and potassium fertilisers and more of these cations were taken up by the plants (although increased calcium uptake may have been related to addition of calcium in the concentrated superphosphate). Their study provides an example of how the application of one mineral nutrient can affect the plant uptake, and hence the grain mineral content of, not only the mineral applied, but others also. An experiment conducted in solution culture by Goodroad *et al.* (1989) showed increased sulphur, nitrogen, magnesium, copper and iron content of the plant tops of soft red winter wheat in response to increased sulphur application. Over the same range of treatments, calcium, manganese and zinc contents decreased. However, they attributed these decreases to a growth dilution effect. Although these changes have been observed in the plant tops, it is likely that at least some of these minerals would be translocated to the grain, possibly resulting in the same changes in mineral concentration being observed in the grain.

Singh *et al.* (1990) applied various rates of both boron and zinc to wheat and found that, although the concentration and content of each mineral in the plant increased as the respective application rates increased, boron concentration and content were reduced in response to increased zinc application rates. Similarly, increased zinc application resulted in lower plant phosphorus content (Singh *et al.*, 1990). The converse is also true: heavy phosphorus application has been shown to induce zinc deficiency in wheat by reducing zinc uptake into the foliage and grain (Sharma *et al.*, 1968; Ragab, 1980; Singh *et al.*, 1986; Choudhary *et al.*, 1995).

This interaction between phosphorus and zinc is widely reported, but little understood. There are various theories to explain the phosphorus-zinc interaction and there could be, likewise, multiple mechanisms active within this interaction. Some researchers have shown that, although the phosphorus-zinc interaction is partly due to dilution of zinc, under low zinc and high phosphorus conditions phosphorus becomes toxic. This is because zinc deficiency results in greater phosphorus uptake and, although zinc concentration is no different to that under low zinc, low phosphorus conditions, symptomatology resembles zinc deficiency (Loneragan *et al.*, 1979; Loneragan *et al.*, 1982). This explanation does not, however, account for the reduction in zinc uptake. Other researchers have shown that the interaction between phosphorus and zinc does not occur at the root-soil interface with phosphorus inhibiting zinc absorption, but rather with phosphorus interfering with zinc translocation from root to shoot (Sharma *et al.*, 1968; Ragab, 1980; Van Steveninck *et al.*, 1993).

Singh *et al.* (1986), however, favoured the external mechanism theory. They agreed with Loneragan *et al.* (1982) that the reduced zinc concentration was partly due to dilution because of growth enhanced by phosphorus fertiliser. However, they also observed that the zinc uptake and concentration of wheat in a phosphorus rate experiment were closely related to the colonisation of the plants by Vesicular Arbuscular Mycorrhizal (VAM) fungi and hence hypothesised that the phosphorus-zinc interaction was partly attributable to the effect of phosphatic fertiliser on VAM infection.

Colonisation of plants by VAM is a symbiotic association between the two organisms. These associations cost the plant in terms of carbon resources but in return the plant receives nutrients. VAM increase nutrient uptake by increasing the plant's effective root soil contact through fine hyphal networks. Various studies have shown that VAM can increase the plant uptake of phosphorus, zinc, copper, manganese and iron under certain conditions (Tinker, 1984; Kucey and Janzen, 1987; Thompson, 1990; Al-Karaki *et al.*, 1998), particularly in soils deficient of these nutrients.

VAM colonisation and inoculum potential have been shown to be affected by crop rotations. *Brassica* crops in the rotation are known to reduce colonisation by VAM in the subsequent crop due to the production of biocidal isothiocyanates (Ryan *et al.*, 1999).

The application of phosphorus fertiliser, either as synthetic phosphate (Thompson, 1990; Ryan and Ash, 1999) or as manure (Tarkalson *et al.*, 1998), results in reduced colonisation by VAM. Under these nutrient sufficient conditions, plants do not need to expend photosynthates in order to increase nutrient acquisition through symbioses with VAM.

It is thought that low phosphorus status in the plant is associated with the mechanism by which plants attract VAM for colonisation. It has been shown that low phosphorus concentration in the root results in greater permeability of root membranes due to a reduction in the levels of phospholipids. This, in turn, results in increased leakage of amino acids and sugars which attract the fungus and initiate root infection (Ratnayake *et al.*, 1978; Graham *et al.*, 1981). VAM formation does not appear to be associated with the release of one particular compound because no substance is consistently present in root exudates of VAM-forming plants and consistently absent in root exudates of non-VAM-forming species (Schwab *et al.*, 1984).

Several researchers have also found VAM colonisation to be greater in organically managed and other low input systems where soil available phosphorus status is low and water soluble phosphatic fertilisers are not used (Ryan *et al.*, 1994; Douds *et al.*, 1995; Dann *et al.*, 1996; Ryan, 1998; Eason *et al.*, 1999; Ryan and Ash, 1999; Mader *et al.*, 2000). Ryan *et al.* (1994) simulated isolated conventional practices in a pot study using organic soil in order to determine which conventional practices most affect the colonisation of VAM. The only practices that resulted in a significant reduction in VAM colonisation were those that included the addition of phosphorus fertiliser.

Other soil micro-organisms have also been shown to affect plant mineral nutrient uptake and hence tissue composition. Rodelas *et al.* (1999) reported that inoculation with bacteria of the genera *Azotobacter* resulted in an increase in calcium, magnesium, potassium, phosphorus, iron and boron contents of faba bean shoots and certain strains resulted in an increase in zinc, manganese and copper contents. Bashan *et al.* (1990) inoculated wheat with the bacteria *Azospirillum brasilense* and found inconsistent variation compared to the uninoculated control with respect to shoot mineral content. However, certain strains of the bacteria significantly altered ion ratios in the plant tissue.

These ratios included magnesium/potassium and sulphur/potassium. Although these results are indirect, they indicate that soil bacteria can influence mineral nutrient uptake in wheat.

Because soil micro-organisms are known to affect the grain mineral composition of wheat, the environmental factors involved immediately multiply and become more complex. For example, the application of farmyard manure was shown by Tarkalson *et al.* (1998) to reduce the percentage of wheat roots colonised by VAM fungi and the same effect was observed after the application of phosphorus to soil from an organic farm (Ryan *et al.*, 1994). Therefore, although these amendments supply additional nutrients for the plant, the grain mineral composition is also affected by their adverse effects on beneficial soil micro-organisms.

VAM fungal colonisation is also reduced in drought conditions (Ryan and Ash, 1996; Al-Karaki *et al.*, 1998). Therefore, as well as influencing grain mineral content through reduced yield and hence concentration of nutrients, drought reduces the plant mineral uptake by reducing VAM fungal associations.

Use of pesticides can also influence grain mineral content by altering microbial populations in the soil. Pesticides applied by Taiwo and Oso (1997) at twice the recommended rates were shown to dramatically change the microbial population dynamics. Summarised, after pesticide application, a short term peak of bacteria and actinomycetes occurred and then dropped back to equilibrium whereas fungal populations rebuilt to equilibrium more slowly and without an initial peak.

They also showed that microbial isolates from soil treated with pesticide were less able to solubilise rock phosphate (Taiwo and Oso, 1997). This is an important action of soil microbes because it mobilises soil minerals into plant available forms and hence increases plant nutrient uptake. The use of pesticides will also result in a short-term nutrient flush as microbial victims are broken down and nutrients released.

The application of herbicides with the active ingredients chlorsulfuron (McLay and Robson, 1992; Osborne *et al.*, 1993; Wheal *et al.*, 1998), diclofop-methyl (McLay and Robson, 1992; Osborne *et al.*, 1993) or metsulfuron-methyl (Pederson *et al.*, 1994) have been shown to reduce the uptake by wheat of nutrients including zinc, manganese, copper, potassium, sulphur and phosphorus. It is thought that reducing root growth, and therefore soil exploration, is the main means by which these herbicides reduce nutrient uptake (McLay and Robson, 1992; Osborne *et al.*, 1993; Pederson *et al.*, 1994; Wheal *et al.*, 1998). Nutrient uptake is not reduced in solution culture where nutrients can freely diffuse to the roots (McLay and Robson, 1992). The effect of herbicides on nutrient uptake has also been shown to diminish with time after herbicide application and as the plants develop (Osborne *et al.*, 1993), except in soils where, for example zinc (Osborne and Robson, 1992), is growth limiting. Furthermore there seems to be no effect of herbicide on grain nutrient composition (Osborne *et al.*, 1993).

Genetic effects on mineral composition

Mineral composition of plant products varies between genotypes, indicating a heritable trait or traits that influence mineral uptake. This genetic effect has been observed in the mineral content of both herbage (shown in ryegrass, (Easton *et al.*, 1997), wheat (Marcar and Graham, 1987; Graham *et al.*, 1992), barley and triticale (Marcar and Graham, 1987)) and grain (shown in wheat (Graham *et al.*, 1992; Grewal and Graham, 1994), barley (Graham *et al.*, 1992) and triticale (Cooper *et al.*, 1988)).

Plants that yield well despite low levels of nutrient in the soil are termed nutrient 'efficient'. Certain wheat genotypes are very nutrient efficient: For example, the variety Molineux appears to be zinc efficient and Janz a manganese efficient genotype (Grewal and Graham, 1994) and conversely, some genotypes are inefficient, for example, Yallaroi is both zinc and manganese inefficient (Grewal and Graham, 1994).

In practice, efficiency is expressed as the ratio of yields obtained when the same genotype is grown under deficient and sufficient conditions (for example, zinc efficiency ranking of a variety is determined by the ratio of its yields without added zinc and with zinc fertiliser applied, both grown in zinc deficient soil). Theoretically, efficiency could be due to the superior ability of a genotype to take up nutrients from the soil or to the superior ability of a genotype to utilise the deficient nutrient. Evidence presented by Rengel and Graham (1996) indicates that at least part of zinc efficiency is due to the plant's ability to take up more zinc. They showed that a zinc efficient wheat variety had a much lower transport of iron from roots to shoot than a zinc inefficient variety when grown in solution culture. Their resulting hypothesis was that zinc uptake is increased in zinc efficient varieties by zinc and iron binding phytosiderophores which are released in response to zinc or iron deficiency.

Rengel and Graham (1996) also reported that the rate of manganese uptake by a zinc inefficient wheat variety was greater when grown with sufficient zinc compared to zinc deficient conditions. However, the opposite was true in a zinc efficient variety.

It has been shown that for wheat grown on manganese deficient soil, efficiency of varieties had a similar ranking regardless of whether efficiency was determined using yield data or grain manganese content data (Grewal and Graham, 1994). This indicates that genotypic manganese efficiency is either caused by superior manganese uptake, the same conclusion made by Rengel and Graham (1996) with respect to zinc efficiency, or is simply an indirect effect of the superior manganese uptake. The question of whether superior nutrient uptake is a cause or effect of nutrient efficiency has not been completely answered yet, but regardless of the outcome, a relationship between genotype and nutrient uptake appears to exist.

Some breeders have attempted to increase the nutrient efficiency of cereals with some success by using efficient parents and selecting progeny on nutrient deficient sites.

The ability to take up minerals of low mobility is increased when a plant has greater root-soil contact. One way of increasing root-soil contact is through greater number of root hairs or by longer root hairs. Different wheat varieties grown under the same conditions have been shown to vary greatly in their root hair surface area, a measurement accounting for both length and number of root hairs (Gahoonia *et al.*, 1997). They showed that a linear relationship exists between the amount of phosphorus removed from the soil by a wheat plant and its root hair surface area. Therefore, the genetic capacity of a variety to produce root hairs is also likely to affect the mineral composition of the plant tissues (including the grain).

The influence of these inherited traits (nutrient efficiency and root hair growth) on mineral uptake indicates that the mineral composition of the grain is determined, at least to some degree, by the genetic make-up of the plant.

2.4.3 Protein and Amino Acids

Protein is another important dietary constituent, of which amino acids are the compositional units. Amino acids must be present in the human body in specific proportions so that protein can be produced efficiently.

Out of the 20 amino acids required by humans, 9 cannot be synthesised by humans and therefore must be obtained through diet. These amino acids are termed essential amino acids and include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, plus histidine for infants (FAO/WHO, 1973). Excess amino acids are converted to fat or to non-essential amino acids (Simmonds, 1989), therefore a balanced intake of amino acids is important.

Cereal grains are characteristically high in glutamic acid, proline, leucine and valine and low in lysine, isoleucine and tryptophan (Simmonds, 1989). Additionally, not all lysine detected through chemical analyses is necessarily available biologically (FAO/WHO, 1973).

A number of researchers (Sosulski *et al.*, 1963; Dubetz and Gardiner, 1979; Miller *et al.*, 1996) reported the amino acid composition of whole wheat grain. Table 2.4 shows the deviation of essential amino acid ratios in wheat from the estimated amino acid ratios required by healthy adult humans (FAO/WHO, 1973). The only amino acids included which deviate more than five percent from the estimated requirements for an adult human are leucine, which is in excess, and lysine, which is low.

Although Table 2.4 indicates that isoleucine is the second limiting amino acid after lysine, Silano *et al.* (1981) present data indicating that, in wheat, threonine is the second limiting amino acid. The calculations by Silano *et al.* (1981), however, are based on the estimated amino acid requirements for children who need a greater proportion of threonine than adults (FAO/WHO, 1973).

Wheat grain protein is divided into four different fractions: albumins, globulins, gliadins and glutenins. Storage proteins contain a high proportion of gliadins and glutenins. These are the protein fractions that are important for bread-making quality (Lasztity, 1984). Bread-making quality includes such characteristics as loaf volume, dough extensibility, dough strength and gluten content. The cytoplasmic proteins (those involved in metabolism) contain a high proportion of albumins and globulins. Albumins and globulins contain more of the essential amino acids lysine, threonine and valine than gliadins and glutenins whereas gliadins and glutenins contain more glutamate (Dubetz *et al.*, 1979). Less than 20 percent of the total endosperm proteins consist of albumins and globulins (Lasztity, 1984) and yet about 80 percent of the total protein present in a wheat grain is found in the endosperm (Vogel *et al.*, 1976).

Environmental effects on protein and amino acid composition

There are many environmental and cultural factors that will alter protein concentration of wheat grain. Ranhotra *et al.* (1996) reported variation in both protein concentration and lysine concentration in the protein of wheat varieties when grown at a number of different locations.

The cultural technique which appears to have the most influence on protein concentration is the application of fertiliser. Abrol *et al.* (1971) compared high and low fertility treatments (including nitrogen, phosphorus and, in the high fertility treatment, potassium) and obtained a higher protein concentration in the grain of wheat grown under high fertility.

The direct response of grain protein concentration in wheat to nitrogen application has been shown by many researchers (Sosulski *et al.*, 1963; Hojjati and Maleki, 1972; Dubetz and Gardiner, 1979). The increase of protein in response to nitrogen fertility can be mostly attributed to an increase in storage proteins, reflected in a corresponding increase in gliadin and glutenin protein fractions (Abrol *et al.*, 1971). However, the amino acid compositions of the individual protein fractions do not appear to alter in response to nitrogen fertiliser (Dubetz *et al.*, 1979).

Table 2.4 Amino acid composition of wheat grain in three surveys with respect to the fulfilment of the estimated human adult amino acid requirement, each expressed as ratios based on g of amino acid/100g protein.

Amino Acid	% of Total EAA* Required by Adult Human (FAO/WHO, 1973)	% of Total EAA Composition in Wheat (Sosulski *et al.*, 1963)	Deviation from Adult Human Requirement	% of Total EAA Composition in Wheat (Dubetz and Gardiner, 1979)	Deviation from Adult Human Requirement	% of Total EAA Composition in Wheat (Miller *et al.*, 1996)	Deviation from Adult Human Requirement
Isoleucine	18.9	18.4	-0.4	15.1	-3.7	15.4	-3.5
Leucine	26.4	31.6	+5.2	32.2	+6.2	31.2	+4.8
Lysine	22.6	14.0	-8.9	16.6	-6.3	17.3	-5.3
Threonine	13.2	12.5	-1.0	14.1	+0.6	15.4	+2.2
Valine	18.9	23.5	+4.7	22.0	+3.2	20.8	+1.9

*Essential amino acids (EAA) for a human adult minus methionine, tryptophan and phenylalanine due to incomplete data.

The amino acid composition of the grain as a whole, however, will change as protein increases, as a result of the increase in proportion of those protein fractions which contain the lowest composition of many of the essential amino acids. Nitrogen application, therefore, has been reported to increase protein concentrations of glutamate (the salt of glutamic acid), proline and also phenylalanine at higher nitrogen levels, but reduce protein concentrations of threonine, serine, glycine and valine (Dubetz *et al.*, 1979). Dubetz and Gardiner (1979) reported a significant negative correlation between the content of glutamic acid and proline (as a percentage of total protein) and most other amino acids. Lysine, histidine and threonine have the highest negative correlation coefficients.

The results of Hojjati and Maleki (1972) show that although the amount of lysine and methionine in the whole grain increase with increasing nitrogen application as total protein concentration increases, the concentration of these amino acids in the protein fluctuates a little but remains relatively stable as protein increases. However, with increasing applications of potassium fertiliser, they reported an increase in the lysine concentration in the protein, particularly evident when no nitrogen was applied. Fertiliser regime, therefore, can alter amino acid composition.

Results of Sosulski *et al.* (1963) show the influence of the soil moisture regime on grain protein concentration in wheat. The highest grain protein was achieved when the soil reached permanent wilting point each time before water was added to bring it back to field capacity. The other treatments were watered back to field capacity long before permanent wilting point was reached. These produced grain with a protein concentration as little as half that of the highest protein. As protein concentration increased, glutamic acid and proline composition of the protein significantly increased but alanine, arginine, aspartic acid, lysine, serine, threonine and valine compositions decreased. This indicates that in the field, both the amount of rainfall and its distribution over time are important in determining the protein concentration and amino acid composition of the grain.

The temperature at which plants are grown post-tillering will also affect the protein concentration of the grain. Sosulski *et al.* (1963) reported that the protein concentration of flour (after milling) increased progressively as the air temperature at which the plants were grown post-tillering increased from 17-24°C. For this reason, delayed sowing could result in an increased protein concentration.

The presence of weeds also affects grain protein concentration but in a complex interaction that reflects competition between the crop and the weeds for nitrogen and water (Mason and Madin, 1996). At sites where soil moisture is the limiting factor, weeds will contribute to an increase in grain protein due to reduced growth and hence a concentration effect. However, at sites where low nitrogen is limiting growth, weeds will compete for the nitrogen and therefore reduce the protein in the grain.

Genetic effects on protein and amino acid composition

When different wheat cultivars are grown at the same location, with the same treatments, there are variations between the cultivars in the protein concentration of the grain produced (Vogel *et al.*, 1976; Ranhotra *et al.*, 1996) and the flour milled from it (Peterson *et al.*, 1983). This indicates a genetic influence on protein concentration. However, this influence may not be directly affecting protein concentration but may be more correctly an effect on yield, which seems to vary inversely to protein concentration.

Lysine has been the main amino acid investigated when wheat cultivars have been compared. Ranhotra *et al.* (1996) reported no significant difference in lysine concentration of protein between different cultivars of wheat or between spelt and common wheat. However, Vogel *et al.* (1976) show the lysine concentration of protein in whole wheat to vary among four cultivars. Surprisingly, this variation has no correlation, negative or positive, with the reported protein concentrations.

There have been attempts to increase the lysine content of wheat through selective breeding. However, although there has been success in breeding high lysine maize (Raloff, 1988; Pixley and Bjarnason, 1994), similar attempts in wheat have been unsuccessful because varieties with superior lysine content tend to have inferior grain protein concentration. This is due to the fact that protein increases are mainly reflected in storage type proteins which are naturally low in lysine (Simmonds, 1989).

2.4.4 Phytate

Myo-inositol hexakisphosphoric acid, phytic acid, and its salt phytate are organic forms of phosphorus found mostly in the seeds of plants. Cereal grains are especially high in phytate which comprises 70 - 90% of the total phosphorus in the whole grain, depending on the species (O'Dell *et al.*, 1972). Wheat has greater phytate concentration than corn, however, soybean has even greater phytate than these cereals (Jan *et al.*, 1985). In wheat 87% of the phytate in the grain is present in the aleurone layer, although there is a similar concentration of phytate in the smaller germ (O'Dell *et al.*, 1972) and it is primarily in the form of the potassium-magnesium salt (Batten *et al.*, 1994).

Phytate is important nutritionally as an 'anti-nutrient'. Phytic acid converts dietary calcium into insoluble, and therefore unabsorbable, calcium phytate. This precipitate, in turn, binds to zinc and iron, rendering these trace elements also unavailable (Wise, 1995).

Because phytate compounds reputedly have low bioavailability, and because of the high proportion of grain phosphorus in the phytate form, phytate is responsible for the large phosphorus content of non-ruminant manures. Therefore, the phytate content of grain can be related to the eutrophication of waterways by effluent (Stelljes, 1996; Ertl *et al.*, 1998; Lott *et al.*, 2000). This is a major environmental problem in Europe and North America.

However, phytate has also been shown to have positive health effects: firstly, to reduce the risk of both colon and mammary cancer (Thompson and Zhang, 1991) and, secondly, to reduce the growth rate of tumours (Jariwalla *et al.*, 1988).

Cultural effects on phytate content

The effects of fertiliser treatments, including combinations with green manure, on the phytate concentration in wheat grain were investigated by Bains (1949). It was found that, although phytate concentration increased, the ratio of phytate to non-phytate phosphorus varied in response to the fertiliser treatment. Each of the chemical fertiliser treatments (without green manure) resulted in either an increase or no change in the proportion of total phosphorus present as phytate (Bains, 1949).

When green manure was included with either no fertiliser, potassium nitrate or superphosphate, the proportion of total phosphorus in the form of phytate was lower than in the equivalent treatment minus green manure. A combination of superphosphate and ammonium nitrate fertilisers was the only chemical treatment in which the addition of green manure did not decrease the proportion of total phosphorus in phytate form (Bains, 1949). It was shown more recently that the proportion of total phosphorus in phytate form increased as the application of phosphatic fertiliser increased (Barrier-Guillot *et al*, 1996). This phenomenon is thought to be due to plants storing excess phosphorus in the form of phytate.

Bassiri and Nahapetian (1979) compared phytate concentrations in wheat grown under irrigated and non-irrigated conditions. They found that when irrigated, both phytate concentration and the proportion of total phosphorus in the form of phytate increased in the wheat grain, whereas the proportion of non-phytate phosphorus decreased.

These studies indicate that fertiliser regimes and farming systems can influence the phytate concentration and proportion, with respect to non-phytate phosphorus, in the grain.

Genetic effects on phytate content

Genotypic variation of phytate concentration in wheat, ranging up to almost 50 percent of the population mean, was reported by Raboy *et al.* (1991). Other researchers, however, reported only low genotypic variance in wheat when assessed for phytate concentration and phytate proportion (Bassiri and Nahapetian, 1979; Barrier-Guillot *et al*, 1996). Similarly, O'Dell *et al.* (1972) found no significant differences in phytate concentration, proportion of total phosphorus and distribution between two corn varieties.

However, genetic mutation technology has been used to produce low phytate mutants of corn, barley and rice (Stelljes, 1996; Lott *et al.*, 2000), indicating that greater genotypic variation in phytate content of wheat may soon be available.

Phytase is an enzyme involved in the digestion of phytate. While phytase is present endogenously in most seeds, wheat has one of the highest phytase activities (Eeckhout and DePaepe, 1994). Barrier-Guillot *et al.* (1996) reported that genotypic variation existed in the endogenous phytase activity in wheat. Variation in phytase activity within the wheat grain is almost as important as variation in phytate in determining the bioavailability, firstly of phosphorus, but also of potassium and magnesium from uncooked grain and of the exogenous dietary calcium, zinc and iron which are complexed by the phytate.

2.5 Bioavailability

It is common for scientific studies to report chemical analyses of foodstuffs in the determination of nutrient content. However, the value obtained as the nutrient content may not accurately reflect the amount of nutrient that can be obtained from a foodstuff when consumed by an animal and is possibly unrelated to the true nutritional value of the food.

Bioavailability, or biological availability, is defined as 'the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilised in metabolism by the animal' (Ammerman *et al.*, 1995, p. 1). The bioavailability of nutrients in a diet is a more accurate assessment of its nutritional value than the nutrient content.

Multiple factors affect bioavailability of nutrients but this literature review focuses on gastrointestinal factors, which are probably the best understood, and their effect on mineral bioavailability.

The most basic factor affecting bioavailability of minerals is the chemical form in which the mineral is ingested. With iron, for example, haemoglobin is more readily absorbed, and hence more bioavailable, than inorganic forms of iron and ferrous iron is more bioavailable than ferric iron (Linder and Munro, 1977, cited by Burk and Solomons, 1985). However, the opposite is true for phosphorus, particularly in non-ruminants, where inorganic forms have greater bioavailability than phytate, an organic form of phosphorus (Soares, 1995). This variation can be partly attributed to the interactions which occur in the intestine between organic compounds and minerals. This is a major factor causing variation in bioavailability.

A much more complex factor affecting bioavailability of minerals is the interaction between the mineral in question and other compounds or minerals ingested at the same time. Phytate is an organic compound often present in diets that reduces bioavailability of minerals including zinc and iron in monogastric animals. However, the effect of phytate on mineral bioavailability has been suggested to depend on its concentration with respect to that of the mineral in question (Morris and Ellis, 1980) and also with respect to calcium concentration (Fordyce *et al.*, 1987). Hence, ratios of phytate to zinc and phytate and calcium to zinc have been developed to assess bioavailability based on analytical results (Morris and Ellis, 1980; Fordyce *et al.*, 1987). Other researchers claim that total phytate is just as accurate as concentration ratios in assessing bioavailability of minerals (Saha *et al.*, 1994).

Phytate supplementation has been shown to increase significantly the amount of faecal zinc excretion (ie. zinc not absorbed from the diet) in rats at varying levels of dietary zinc (Davies and Nightingale, 1975; House *et al.*, 1982). This was reflected in reduced bone and blood plasma zinc concentrations, which are used as indications of zinc bioavailability, and also in kidney zinc concentrations (House *et al.*, 1982). Endogenous zinc (ie. zinc already present in the body) excretion in rats was also increased by phytate supplementation and this was also reflected in lower bone zinc concentration (Flanagan, 1984). Based on whole body mineral contents in rats, Davies and Nightingale (1975) demonstrated that phytate supplementation reduces the bioavailability of iron, copper and manganese as well as zinc.

Saha *et al.* (1994) showed that endogenous dietary phytate (ie. phytate present in the unsupplemented diet) also reduces the absorption of zinc, iron and selenium by rats. High endogenous phytate (as opposed to medium high phytate) also reduced the absorption of calcium by rats (Saha *et al.*, 1994).

Minerals also interact with each other to alter bioavailability. Often this occurs when minerals are involved in the same metabolic pathway which can then be saturated by an excess of a single mineral, reducing the bioavailability of other minerals. Alternatively, mineral bioavailability can be reduced through excess of a mineral of similar size or charge which can be substituted for the mineral in question in a critical metabolic step.

An example of this is the strong interaction between copper and zinc. Excess zinc will reduce copper bioavailability but extreme excess copper will also reduce zinc absorption (Van Campen, 1969). There is also an interaction between iron and zinc. It is thought that excess zinc could interfere with the production of ferritin (Settlemire and Matrone, 1967). Ferritin is involved in iron storage in the liver and thought to be involved in iron absorption in the small intestine, hence zinc reduces the bioavailability of iron. Interactions between many other metal ions have also been reported. Many of these interactions are competitive and therefore negative.

Other factors are also involved in the determination of bioavailability of a mineral. An example is the bioavailability of phosphorus from grain and seed sources where it is largely present in the form of phytate. In this case, phosphorus bioavailability (and also bioavailability of minerals with which phytate forms insoluble compounds) depends on the presence of phytase, present either in the grain (Pointillart *et al.*, 1987) or as microbial phytase in the gut (Davies and Flett, 1978; Moore and Veum, 1983). Rye, triticale, wheat and barley are relatively rich in phytase but other plant feedstuffs are not (Eeckhout and DePaepe, 1994). The endogenous phytase activity in the grain is independent of the amount of phytate present (Eeckhout and DePaepe, 1994; Barrier-Guillot *et al.*, 1996). Phytase is produced by microbes in the gut of ruminants, however it is produced in much smaller quantities in the gut of monogastric animals (Cooper and Gowing, 1983; Stelljes, 1996).

Bioavailability can also be altered through processing methods. Phosphorus absorption was lower for rats fed raw chickpeas or those soaked in citric acid solution (compared to those soaked in water or sodium bicarbonate solution or soaked and cooked). Heating increased phosphorus absorption from chickpeas (Nestares *et al.*, 1999).

2.6 Summary

A diet that is balanced with respect to minerals, vitamins and amino acids is important in maintaining human health. However, the nutritional value of food is not based on composition alone but on the value of the components to the consumer and this is affected by nutrient interactions.

Claims have been made by consumers and producers of organically grown food that this food is more nutritious than conventionally grown food. Different production systems will potentially affect nutritional composition because there are many cultural factors that cause changes in nutritional composition of food. One cultural factor that has particular effect on the nutritional composition of food is the capacity of the farming system to deliver nutrients to the plant and is related to the application of various kinds of fertilisers.

Studies comparing the composition of organically and conventionally grown food, although most reporting some differences, usually conclude that differences were not consistent. In some cases this was due to poor experimental design but could also be due to a myriad of environmental influences external to the production system that cannot be controlled and also affect the nutritional composition of food. Very few of these studies have used Australian organically grown wheat, for which the cultural methods are quite different to those in Europe, and which therefore have possibly quite different nutrient compositions. Also, there are no studies comparing organically and conventionally grown wheat in areas of low rainfall. Furthermore, no Australian studies have followed up on compositional differences, to compare organically and conventionally grown wheat in feeding trials, although Derrick (1996) suggested it, based on his results.

Chapter 3

Comparison of Organic and Conventional Farming Systems: Growth and Yield of Wheat

3.1 Introduction

The field component of this study was designed to compare the growth, yield and grain nutrient content of wheat grown in organic and conventional systems.

Grain yield in organic systems is often less than grain yields in conventional systems. However, it has been suggested that the comparative yield loss of organic systems is less in dry seasons when the conventional yield is restricted by low rainfall (Wynen, 1994). This presents the possibility, and the hypothesis of this study, that in the wheat belt of South Australia, particularly the areas of low rainfall (less than 350 mm *per annum*), the yields in organic and conventional systems are more similar.

Wheat breeders select varieties that are most adapted to the conditions under which they are grown, for example the dry Australian conditions. It is possible, therefore, that wheat varieties bred in the early twentieth century (ie. prior to widespread fertiliser and chemical use) may be more adapted to the conditions of current organic farming techniques, for example greater weediness and less soluble nutrient supply than in conventional farming systems. Thus the hypothesis emerges that old wheat varieties are better adapted to organic farming techniques than the more recently released varieties.

The aims of the current investigation, therefore, were threefold:

- To compare the growth and yield of wheat in organic and conventional systems under South Australian conditions,
- To assess the importance of rainfall on the growth and yield of organic systems relative to conventional systems and
- To investigate the influence of selected wheat varieties, including old wheat varieties, on growth and yield of organic systems.

Because of the limited time frame of this study and the desire to compare organically and conventionally grown grain that was representative of grain marketed as such, the most appropriate method of systems research (section 2.3.4) was on-farm comparisons.

Three pairs of organic and conventional farms were selected in each of three areas of South Australia: Wolseley (average annual rainfall 470 mm), Nadda (average annual rainfall 274 mm) and Wudinna (average annual rainfall 318 mm). Wolseley was selected because there were a number of organic farms, managed by different farmers, in the same ecological area. These were operated using a number of management types ranging from no inputs at all to the use of a variety of organically acceptable inputs. Nadda and Wudinna were selected because they were areas of low rainfall, contrasting with Wolseley, which is a high rainfall location within South Australia.

3.2 Methods

In both 1997 and 1998, replicated field trials were sown on neighbouring organically and conventionally operated farms at Wolseley (comparisons A-C), Nadda (comparisons D-F) and Wudinna (comparisons G-I) (Figure 3.1, Appendix 1). Each comparison therefore comprised two neighbouring sites, one certified organic by The National Association for Sustainable Agriculture, Australia (NASAA) or Biological Farmers of Australia (BFA), the other farmed conventionally. The experiments were located in paddocks where wheat was to be sown in that year. The need to sow the experiments in the wheat phase of the rotation in each of the farms meant that none of the Wolseley comparisons were directly across the fence from each other, but across the fence comparisons were possible at both Nadda and Wudinna. However, sites at Wolseley were selected based on apparent uniformity of soil type.

Comparisons between the same organic and conventional farms in consecutive years were labelled with the same letter, but with the year specified (eg. comparison A (1997)). However, it is to be noted that in following the wheat phase of the rotations, comparisons in consecutive years were not sown in the same paddocks.

A split plot design was used (as shown in Figure 3.2) with the system treatment making up the whole plots, and wheat varieties randomly distributed in subplots. There were ten replicates, but not all replicates were used for all measurements. Due to the nature of the study, the system treatment could not be randomly applied to the whole plots and therefore, the replicates are essentially pseudo-replicates. Consequently, the statistical analysis is based on the assumption that the system effect is a true treatment effect and not an effect related to one side of the experiment. This assumption is valid if most differences influenced by farming system were greater than pre-existing soil or site differences.

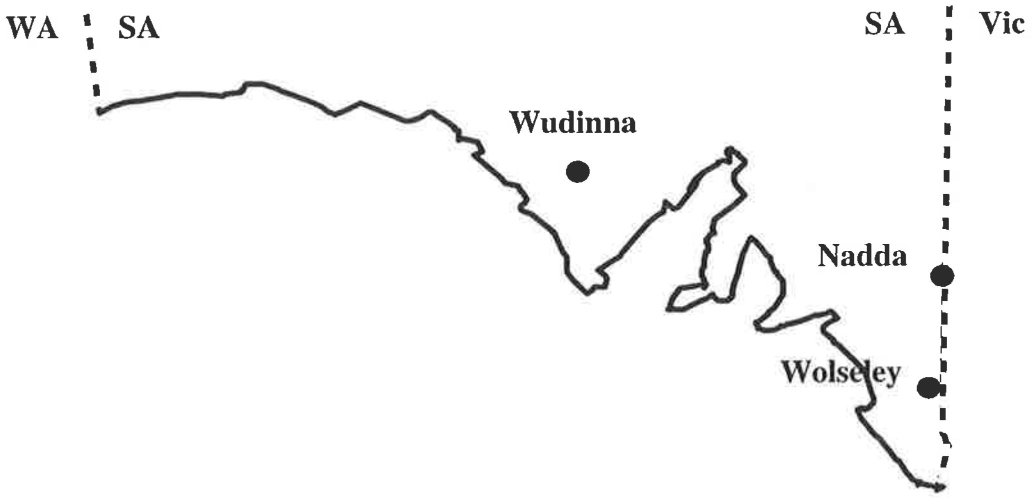


Figure 3.1 Southern South Australia showing the geographical locations of Wolseley, Nadda and Wudinna, the regions of the field trials. Scale 1 cm: 100 km

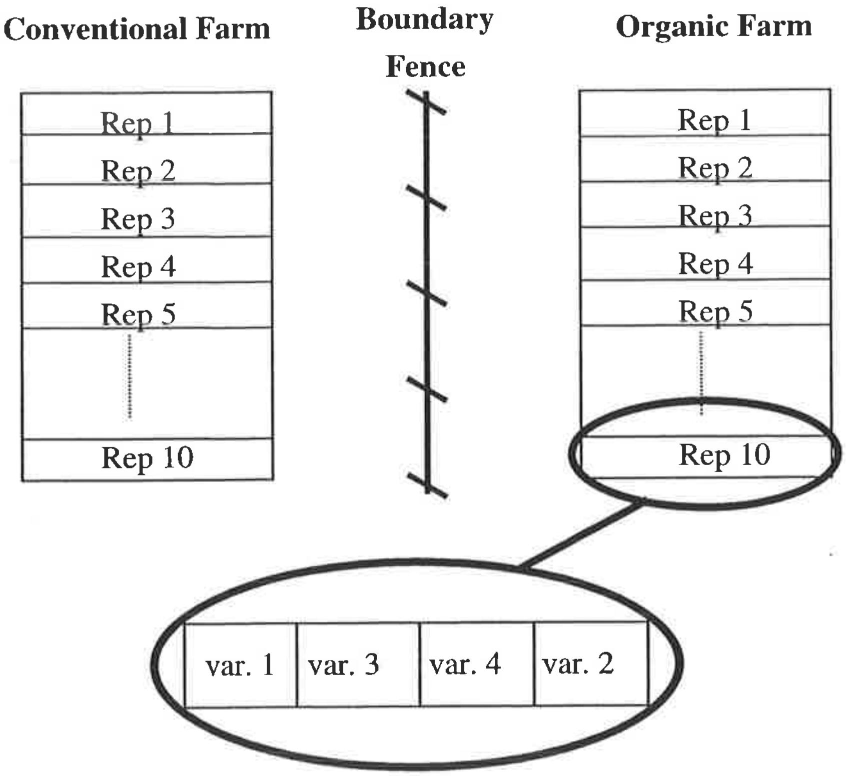


Figure 3.2 A representation of the experimental layout of the field trials at Nadda and Wudinna in 1998 showing randomised varieties (var.) at the subplot level.

Plots at Wolseley and Nadda were sown 6 rows (1 m) wide and 6 m long using a small-plot cone seeder with delivery of seed and fertiliser (where applicable) together to a boot behind each tine. Seeding depth was 25-30 mm and the seeding rate was 190 seeds/m^2. In order to avoid varietal contamination between consecutive plots, the harvested length of the plots was 4.2 m. The plots at Wudinna were set up in the same way, but using different machinery due to logistics. Hence, the plots in the Wudinna comparisons were 8 rows (1.6 m) wide and harvested 5 m long.

Rainfall recorded within the growing season at each of the field localities is shown in Figures 3.3 and 3.4. These rainfall data were recorded at the closest farmhouse to the trial locations.

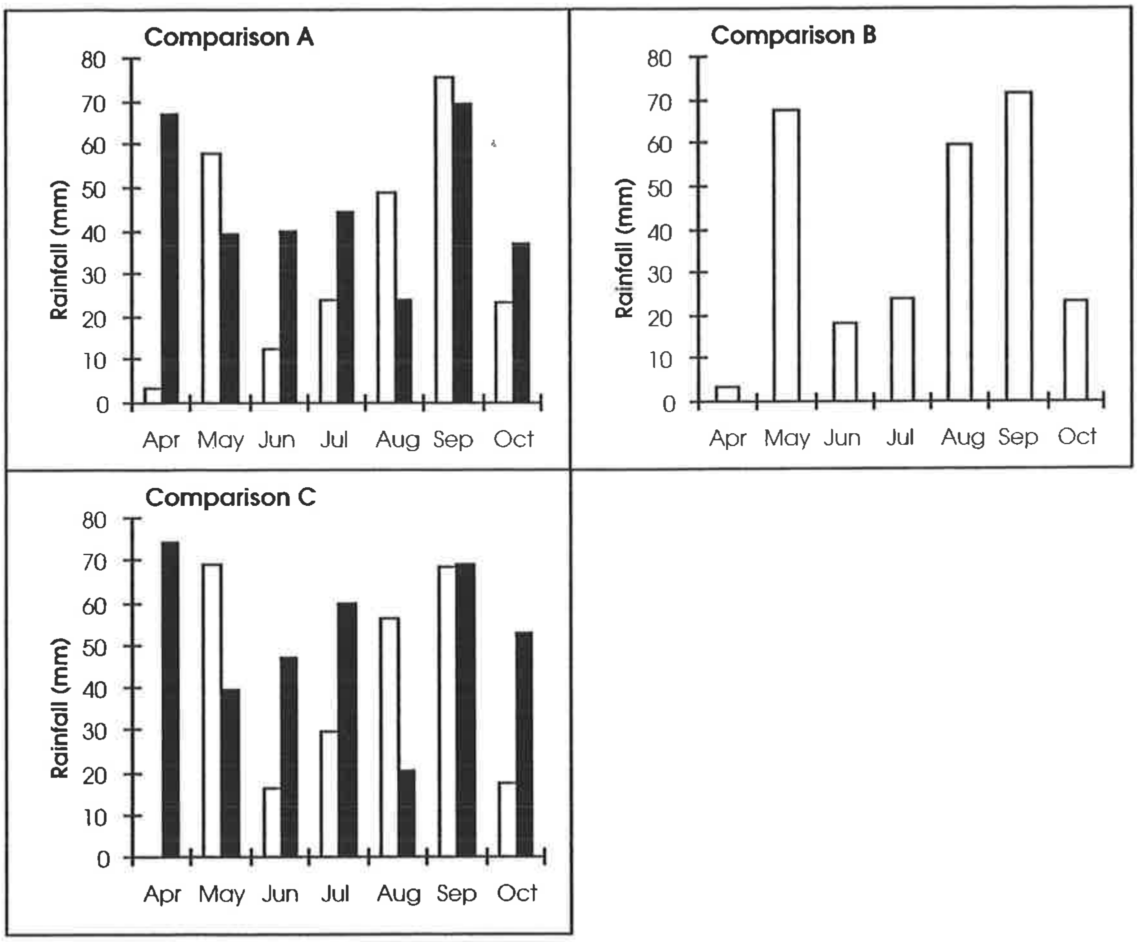


Figure 3.3 Rainfall through the growing season at the high rainfall (Wolseley) comparisons in 1997 (☐) and 1998 (■). April to October rainfall totals are: Comparison A (1997) 246 mm, (1998) 320 mm; Comparison B (1997) 268 mm; Comparison C (1997) 257 mm, (1998) 363 mm.

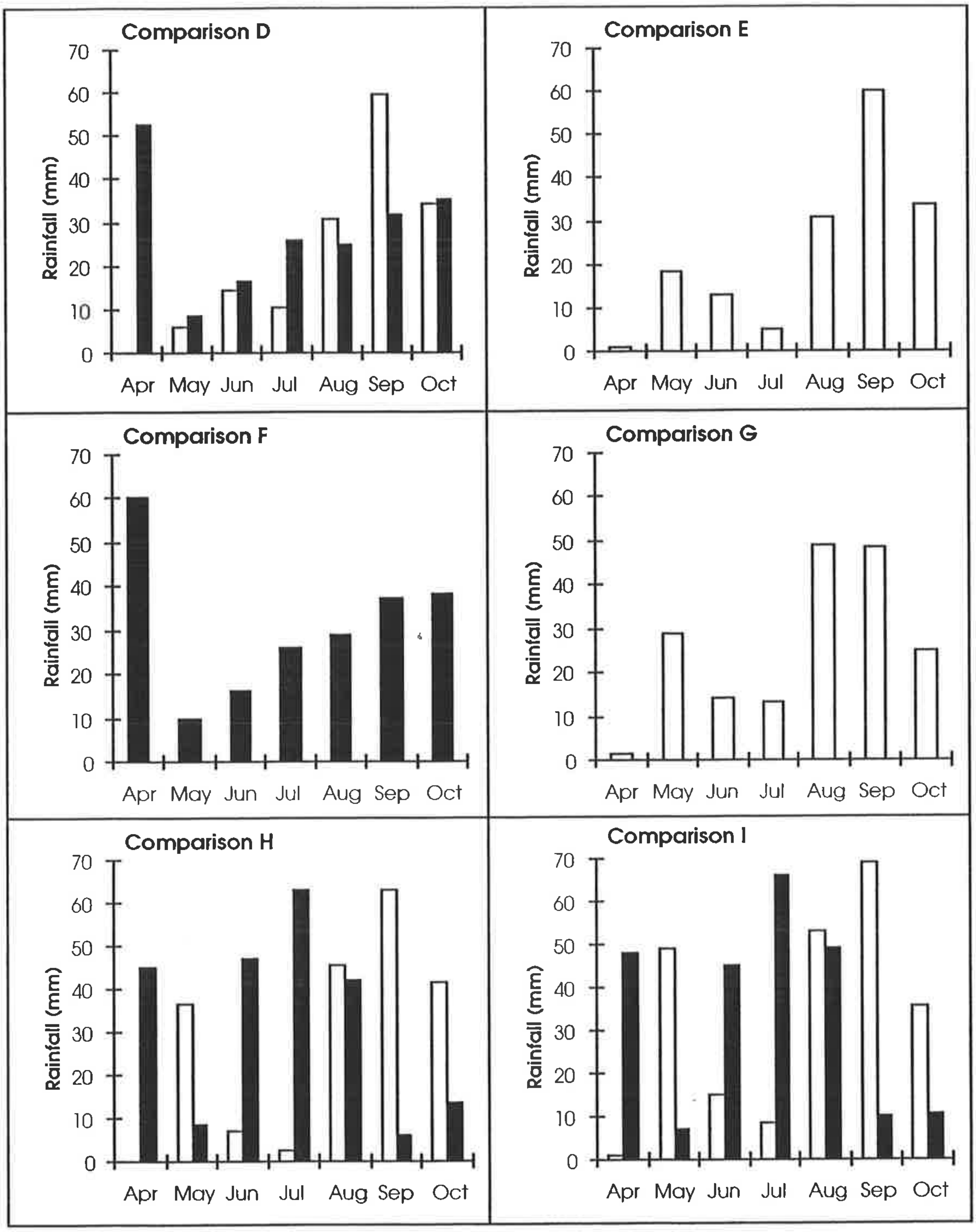


Figure 3.4 Rainfall through the growing season at the low rainfall comparisons (Nadda (comparisons D, E and F) and Wudinna (comparisons G, H and I)) in 1997 (□) and 1998 (■). April to October rainfall totals are: Comparison D (1997) 155 mm, (1998) 195 mm; Comparison E (1997) 163 mm; Comparison F (1998) 216 mm; Comparison G (1997) 180 mm; Comparison H (1997) 196 mm, (1998) 225 mm; Comparison I (1997) 231 mm, (1998) 236 mm.

3.2.1 Wheat Varieties

In order to determine the effect of wheat variety on growth and yield within organic systems, five varieties of wheat (Janz, Trident, Yallaroi, Dirk-48 and Baroota Wonder) were sown in 1997 and four (Janz, Trident, Yallaroi and Dirk-48) in 1998. Janz and Trident were selected because they had been reported as being manganese (Grewal and Graham, 1994) and zinc (Moussavi-Nik, 1997) efficient respectively and Yallaroi, a durum wheat, was included as a nutrient inefficient variety (Grewal and Graham, 1994). Dirk-48 and Baroota Wonder, both tall wheats (released in 1949 and selected in 1895 respectively) were included as wheat varieties selected for low input agriculture. Baroota Wonder was excluded in 1998 owing to its consistently poor yield performance in both farming systems in 1997.

3.2.2 Management

The management of the trial plots grown under the contrasting systems was modelled as closely as possible on that of the wheat surrounding each trial. By doing this, the wheat grown in the experimental plots under each system was closely representative of the wheat that is grown and sold as either organically or conventionally grown. This was an important consideration because grain from the trials would be subsequently used in assessment of nutritional composition and value (see Chapters 4 and 5 respectively).

This management aim was achieved by applying the same fertilisers at sowing as the farmer. Other cultural activities (for example, herbicide or foliar fertiliser applications) were applied by the farmer to the experimental plots within his paddock at the same time that he applied them to the surrounding wheat. A summary of the cultural practices within each system is shown in Tables 3.1 and 3.2. More details are included in Appendix 1. In this way, differences in management between the trial plots and the surrounding wheat were minimised.

An unavoidable difference, however, was the sowing date. The organically and conventionally managed trials were sown on the same day, due to logistics, but this rarely occurs in reality. As a consequence of the adjusted sowing date, the weed control in the organically managed systems was compromised. Furthermore, the plot seeder had narrow points rather than cultivating shears as used by the farmers at seeding and because of this, the experimental plots were not cultivated in the seeding operation. In most conventional systems this made little difference because the weeds were later dealt with using herbicides. In the organic system, however, the final weed kill before sowing is critical in weed management and hence, weeds within the experimental organic systems were usually more dense than in the surrounding crop.

3.2.3 Sampling and Measurements

Soil Testing

The soil in all three Wolseley comparisons was a grey cracking clay (CC9; Northcote, 1960). The Nadda soil was a brown calcareous earth (DD1; Northcote, 1960) and the Wudinna soil was a grey brown, highly calcareous loamy earth (Lb5; Northcote, 1960).

Eight soil cores of 0-10 cm depth were collected in a grid pattern across each trial site in September of each year. The samples from each system at each site were mixed and a single sub-sample was taken for analysis.

The soil was air-dried and analysed by Wesfarmers CSBP Soil and Plant Laboratory (Kwinana, Western Australia) for organic carbon (Walkley and Black, 1934), pH in 0.01M calcium chloride (White, 1969), sodium bicarbonate extractable phosphorus (Colwell, 1963), sulphur (Blair *et al.*, 1991), exchangeable cations (Gillman and Sumpter, 1986) and DTPA extractable trace elements (Lindsay and Norvell, 1978).

Biomass Sampling

In 1997, plant tissue was collected from all plots at late tillering (around developmental stage 25 (Zadoks *et al.*, 1974)) by taking a 50 cm cut in a single interior row. In 1998, samples were collected at late tillering from five replicates only. Additional samples were collected in 1998 (from comparisons A, C, D and F only) in the same way and from the same plots at anthesis (around developmental stage 65 (Zadoks *et al.*, 1974)) and maturity (prior to harvest).

Biomass production was estimated per hectare based on the dry weight of each 50 cm row cut. Grain yield per hectare was estimated based on the grain yield of each experimental plot. In 1998, the grain yield of the plots from which the three biomass cuts were taken, was corrected based on the proportional yield difference between the sampled and unsampled plots.

Weed counts were made based on counts within four $0.25m^2$ quadrats across the trial site and are included as an indication of weediness.

Table 3.1 A summary of the management of 1997 organic (Org) and conventional (Conv) system comparisons.

		Wolseley			Nadda		Wudinna		
Comparison		**A**	**B**	**C**	**D**	**E**	**G**	**H**	**I**
Previous Crop:									
	Org	Fallow	Beans+Vetch	Pasture/Silage	Pasture/Fallow	Pasture/Fallow	Fallow	Pasture	Pasture
	Conv	Peas	Fallow	Peas	Pasture	Pasture	Pasture	Pasture	Pasture
Sowing Date:									
	Org	10 June	10 June	10 June	15 June	15 June	20 June	24 June	24 June
	Conv	10 June	10 June	10 June	15 June	15 June	20 June	24 June	24 June
Fertiliser:*									
N	Org	6.9**+0.5	0	0	0	0	0	0	0
	Conv	18.0	5.6	12.6	5.6	0	8.5	0	0
P	Org	9.6**+2.8	0	14.5**	0.2**	0.2**	0	0	0
	Conv	20.0	13.3	14.0	6.2	7.7	9.5	8.0	9.0
Zn	Org	0.28**	0	0.28***	Trace**#	Trace**	0	0	0
	Conv	0	1.4	0.28***	0	0	2.5	1.82**	0.25***

*Fertiliser (kg/ha) applied at time of sowing unless otherwise indicated **Fertiliser applied pre-sowing

***Mid-season foliar fertiliser #Trace indicates 1-10 g/ha

Table 3.2 A summary of the management of 1998 organic (Org) and conventional (Conv) system comparisons.

	Wolseley		Nadda		Wudinna	
Comparison	**A**	**C**	**D**	**F**	**H**	**I**
Previous Crop:						
Org	Vetch+Oat Hay	Pasture	Pasture/Fallow	Pasture/Fallow	Pasture	Pasture
Conv	Peas	Canola	Pasture	Pasture	Pasture	Pasture
Sowing Date:						
Org	10 June	10 June	27 May	27 May	10 June	10 June
Conv	10 June	10 June	27 May	27 May	10 June	10 June
Fertiliser:*						
N Org	0	0	0	0	0	0
Conv	12.6**	6.0	7.2	7.0	0	0
P Org	20**	10**	0.2**	0.2**	0.2**	0.2**
Conv	14.0**	13.2	8.0	15.4	8.8	12.0
Zn Org	0.07**	0.03**	Trace**#	Trace**	Trace**	Trace**
Conv	0.14***	0	0	0	1.66**	1.2

*Fertiliser (kg/ha) applied at time of sowing unless otherwise indicated **Fertiliser applied pre-sowing

***Mid-season foliar fertiliser #Trace indicates 1-10 g/ha

3.2.4 Statistical Analysis

Grain yield and biomass production were analysed using GENSTAT 5 with a factorial split plot design to calculate the ANOVA (analysis of variance). For this analysis, the farm comparisons (sites) were grouped into locations (ie. Wolseley, Nadda or Wudinna) and each location analysed separately. Each season was also analysed separately. The data were blocked using farm comparison (site) and replicate. The treatments were system and variety. An example of the structure of the ANOVA table is shown in Table 3.3.

Most of the grain yield and biomass production data are presented with the varieties averaged within each farming system. This is valid because, where significant variety by system interactions occurred, the interaction did not involve an inversion of the system effect.

The soil data was based on single replicates of each system within each comparison of the systems. Hence these were analysed in the same way except that the comparisons were treated as replicates of the systems.

Tukey's Multiple Comparison Test was used to determine differences between the varieties in terms of biomass production and yield. Where there were missing values, the Tukey Kramer Test was used.

Table 3.3 An outline of the ANOVA table for biomass at Nadda (1997).

Source of Variation	Degrees of Freedom
Comparison* Stratum	(1)**
Comparison.Rep Stratum	(18)
Comparison.Rep.System Stratum	(20)
System	1
Residual	19
Comparison.Rep.Variety Stratum	(160)
Variety	4
System.Variety	4
Residual	152
Total	199

*The comparison stratum here represents the site stratum, however it is not labelled as site because each comparison, in effect, comprises two sites: organic and conventional.

**Parentheses indicate the total degrees of freedom within a stratum.

3.3 Results

3.3.1 Soil

A summary of the soil test results is shown in Tables 3.4 and 3.5. Further analyses are provided in Appendix 2. There is great variation between the comparisons for most of the soil variables, rendering most differences between the farming systems non-significant.

Differences in pH between the organic and conventional systems were not significant in either high or low rainfall areas.

There was no difference in the organic carbon content of the soil between the organic and conventional systems in the higher rainfall area (Table 3.4). In the areas of low rainfall, however, the soil from the organic systems had greater than or similar organic carbon to that from the neighbouring conventional systems (Table 3.5).

The extractable phosphorus content of the soil was most consistently different between the systems. For the higher rainfall area (Wolseley), the conventional systems had significantly greater extractable soil phosphorus than the organic systems. A similar result was apparent in the low rainfall areas except for three comparisons (D, E and H) in 1997, resulting in non-significance overall.

The differences in the zinc content of the soil were large for some comparisons of organic and conventional systems. However, they were not significant overall for either high or low rainfall areas.

The extractable copper was significantly greater in the soil of the organic systems than the conventional systems from the higher rainfall area. However, there was clearly no difference in soil copper between the two farming systems in the low rainfall areas.

Table 3.4 Summary of the results of the soil tests (0-10 cm) comparing organic (Org) and conventional (Conv) systems under high rainfall conditions (Wolseley) in 1997 and 1998.

Year		**1997**			**1998**			
Comparison		**A**	**B**	**C**	**A**	**C**	**Mean**	**F Prob**
pH (CaCl$_2$)	Org	7.1	7.7	7.6	7.9	7.8	7.6	NS
	Conv	7.7	7.8	7.5	7.7	7.6	7.7	
Organic Carbon (%)	Org	1.24	1.13	1.13	1.50	1.46	1.29	NS
	Conv	1.23	1.39	1.16	1.48	1.54	1.36	
Colwell P (mg/kg)	Org	11	18	13	9	20	14.2	P=0.025
	Conv	26	29	14	33	34	27.2	
DTPA Zn (mg/kg)	Org	0.53	0.66	0.52	0.52	0.57	0.56	NS
	Conv	0.63	1.33	1.06	0.71	0.51	0.85	
DTPA Cu (mg/kg)	Org	0.58	0.57	0.69	0.82	0.84	0.70	P=0.044
	Conv	0.62	0.42	0.52	0.60	0.70	0.57	

Table 3.5 Summary of the results of the soil tests (0-10 cm) comparing organic (Org) and conventional (Conv) systems under low rainfall conditions (Nadda (D, E, F) and Wudinna (G, H, I)) in 1997 and 1998.

Year		**1997**					**1998**					
Comparison		**D**	**E**	**G**	**H**	**I**	**D**	**F**	**H**	**I**	**Mean**	**F Prob**
pH	Org	7.8	7.5	7.9	7.8	7.9	5.9	5.7	8.0	7.5	7.3	NS
($CaCl_2$)	Conv	7.8	7.6	7.8	7.7	7.8	5.8	5.7	7.9	6.9	7.2	
Organic Carbon (%)	Org	0.85	0.74	1.03	2.34	1.87	0.58	0.42	1.70	0.82	1.15	P=0.016
	Conv	0.88	0.63	0.95	1.84	1.30	0.44	0.45	1.40	0.39	0.92	
Colwell P (mg/kg)	Org	15	19	18	23	10	9	10	20	5	14.3	NS
	Conv	16	11	48	22	22	21	25	27	12	22.7	
DTPA Zn (mg/kg)	Org	0.49	0.91	0.65	1.39	0.95	0.28	0.26	0.49	0.24	0.63	NS
	Conv	0.41	0.39	1.39	1.04	0.99	1.01	0.42	0.89	0.21	0.75	
DTPA Cu (mg/kg)	Org	0.33	0.19	0.40	0.60	0.71	0.28	0.23	0.81	0.19	0.42	NS
	Conv	0.32	0.16	0.51	0.49	0.97	0.24	0.32	0.50	0.17	0.41	

3.3.2 Biomass

In both high and low rainfall areas, the conventional systems produced at least as much biomass, and usually more, by late tillering than the organic systems (Figure 3.5). For the one exception (comparison I, 1997) where the organic system had significantly greater biomass than the conventional system at late tillering, the conventional system produced abnormally low early growth for that farming system, as judged by comparing the experimental plots to the surrounding wheat crop.

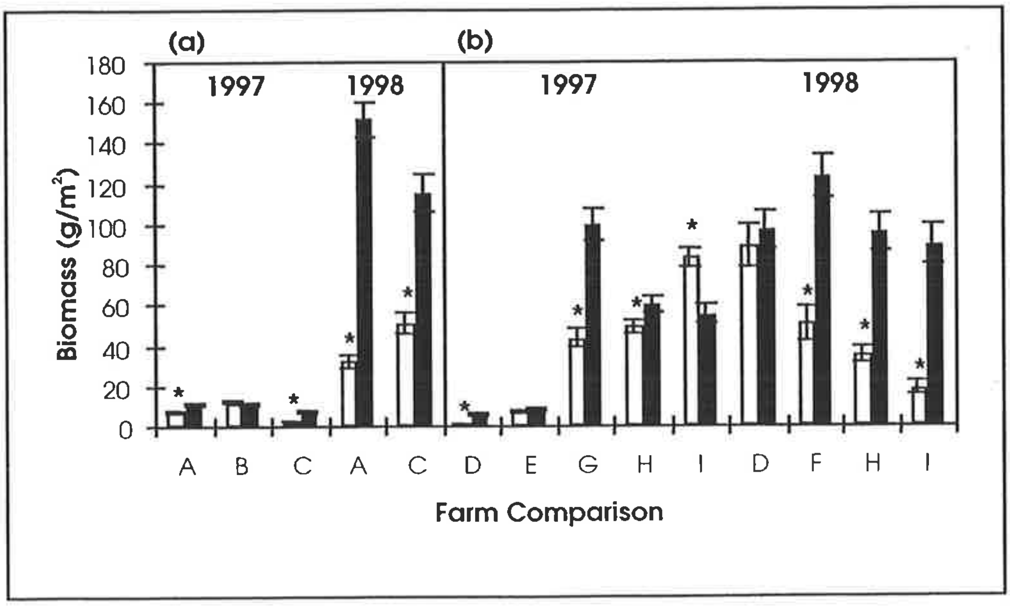


Figure 3.5 Above ground dry weight biomass production at late tillering of wheat grown in organic (□) and conventional (■) systems in high **(a)** and low **(b)** rainfall areas in 1997 and 1998. The asterisk indicates that the organic system is significantly different to the conventional system ($P<0.05$) for that comparison.

The biomass production at late tillering of the selected wheat varieties grown under the organic and conventional systems is shown in Figure 3.6. The results were similar between the high and low rainfall areas. The interaction between system and variety had no significant effect on biomass at late tillering. However, there was a highly significant effect of variety on biomass, with the consistent trend being that the old varieties produced greater biomass. In 1998, measurements of biomass production were made at three developmental stages in comparisons A and C of the high rainfall area and comparisons D and F of the low rainfall areas. For comparisons A and F, the conventional system produced significantly more biomass than the organic system at all developmental stages, whereas comparisons C and D showed no significant difference in biomass production between the farming systems from anthesis onwards (Figure 3.7).

3.3.3 Grain Yield

Both the organic and conventional experimental plots were compared with the surrounding crop and shown (for yield, at least) to be a good approximation of the systems (organic and conventional experimental plots yielded an average of 96.6 and 96.4 percent of the surrounding crop yield respectively when the same variety was compared). However, these comparisons within the organic systems were much more variable (ranging 46-177 percent) than the conventional systems (ranging 80-122 percent) which indicates that the conventional systems were more accurately and consistently approximated by the experimental plots than the organic systems.

There were no comparisons for which the grain yields of the two farming systems were the same (Figure 3.8). With three exceptions, the conventional system produced significantly greater yield than the organic system. The exceptions were the high rainfall comparison A (1997) and the low rainfall comparisons I (1997) and D (1998). However, most of the organic systems in the high rainfall comparisons produced 20 to 30 percent lower yields than the conventional systems, whereas the organic systems in the low rainfall comparisons yielded as much as 72 percent lower than the conventional systems (comparison D, 1997).

There was no overall consistent difference in thousand-grain weight between the organic and conventional systems (Figure 3.9), and for many comparisons there was no difference at all. Where there were significant differences in grain weight between the farming systems for the high rainfall comparisons, the differences were small.

In comparing the grain yield of the five wheat varieties (Figure 3.10), there were only two significant interactions between system and variety: Nadda in 1997 and Wolseley in 1998. These interactions were not consistent with each other and, therefore, there was no consistent indication that any of the varieties tested were better adapted to either the organic or conventional farming system.

Trident yielded highest or equal highest in both organic and conventional systems and in both high and low rainfall areas. Similarly, when Baroota Wonder was included in the comparison in 1997, it yielded lower than or equal to the other varieties. Yallaroi consistently yielded lower than the other varieties (excluding Baroota Wonder), particularly in the low rainfall regions. .

3.4 Discussion

3.4.1 Soil

The results of the soil analyses are discussed in the context of plant nutrition in Chapter 4. The effect of the soil on plant nutrient acquisition is one of the most important ways in which soil properties can influence biomass production and grain yield.

3.4.2 Biomass

The biomass production of the organically grown wheat was less than or equal to the conventionally grown wheat at all developmental stages in all comparisons (excluding comparison I (1997) for which the early vigour in the experimental conventional system did not represent the surrounding system) (Figures 3.5 and 3.6). Because the biomass production of wheat is of less economic importance than the grain, it is not widely reported in the literature for organic systems. However, where it is reported, results similar to the current study have been obtained. Derrick (1996) found the organic system to produce much less biomass than the conventional at the same developmental stages as the current study and in each of three seasons.

The rainfall regime had very little effect on the difference in biomass production between organic and conventional farming systems (Figures 3.5 and 3.6). This reveals that the growth of the wheat in the organic system is not being limited by rainfall any more or less than the growth of wheat in the conventional system.

The tall wheat varieties, Baroota Wonder and Dirk-48, seemed to produce greater biomass than the more recently released, semi-dwarf varieties by late tillering (Figure 3.6). Furthermore, there was no difference between the old and more recent varieties in their relative biomass production between the organic and conventional farming systems, indicating an absence of adaptation by the more recent varieties to the conventional farming systems.

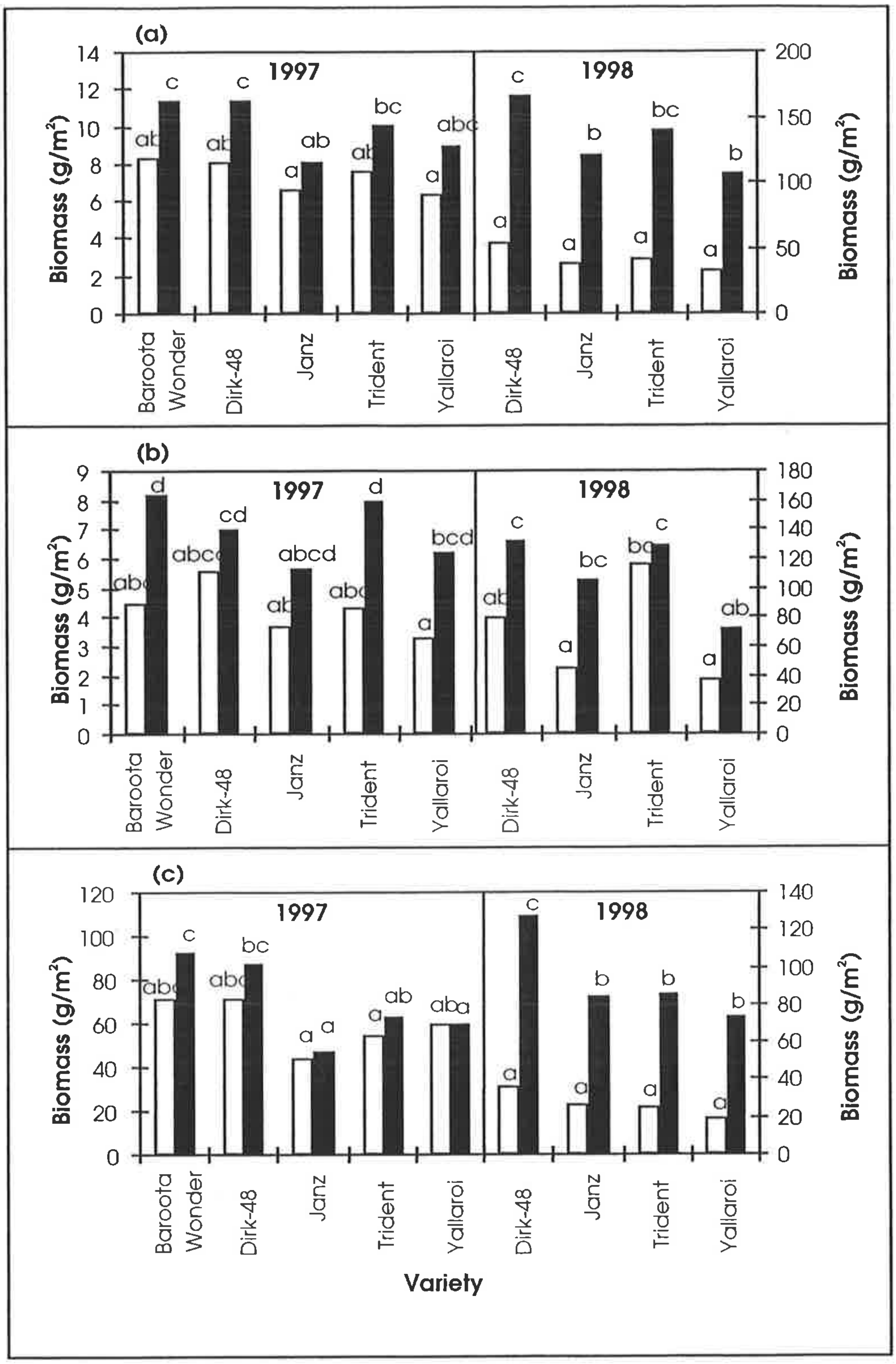


Figure 3.6 Above ground dry weight biomass production at late tillering (average of all farm comparisons) of selected wheat varieties grown in organic (☐) and conventional (■) systems in high (**a**) and low (**b** and **c**) rainfall areas (Wolseley, Nadda and Wudinna respectively) in 1997 and 1998. Columns within the same season and with the same letter above are not significantly different (P>0.05). There was no significant interaction between farming system and variety.

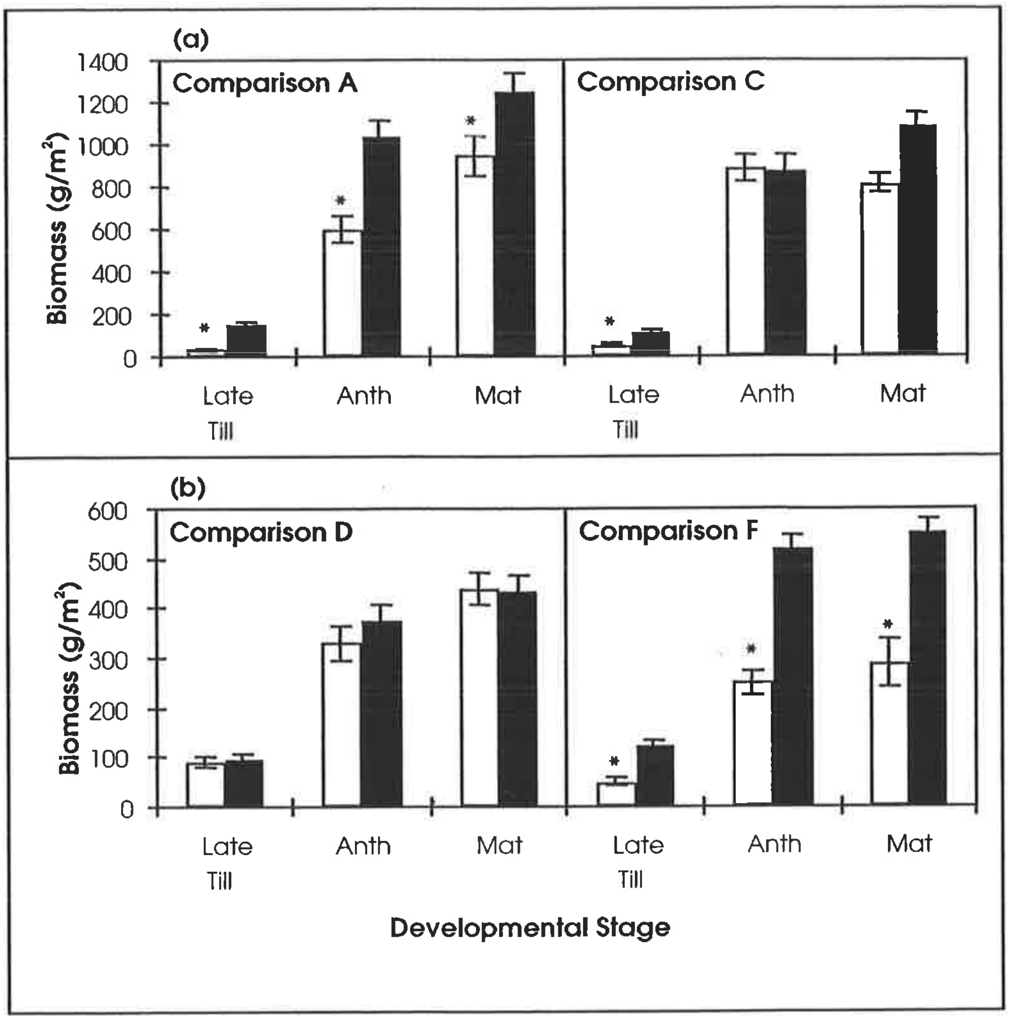


Figure 3.7 Above ground dry weight biomass production of wheat (average of selected varieties) in 1998 organic (□) and conventional (■) system comparisons in high **(a)** and low **(b)** rainfall areas. Measurements were made at late tillering (Late Till), anthesis (Anth) and maturity, including heads of grain (Mat). The asterisk indicates that the organic system is significantly different to the conventional system ($P<0.05$) for that developmental stage.

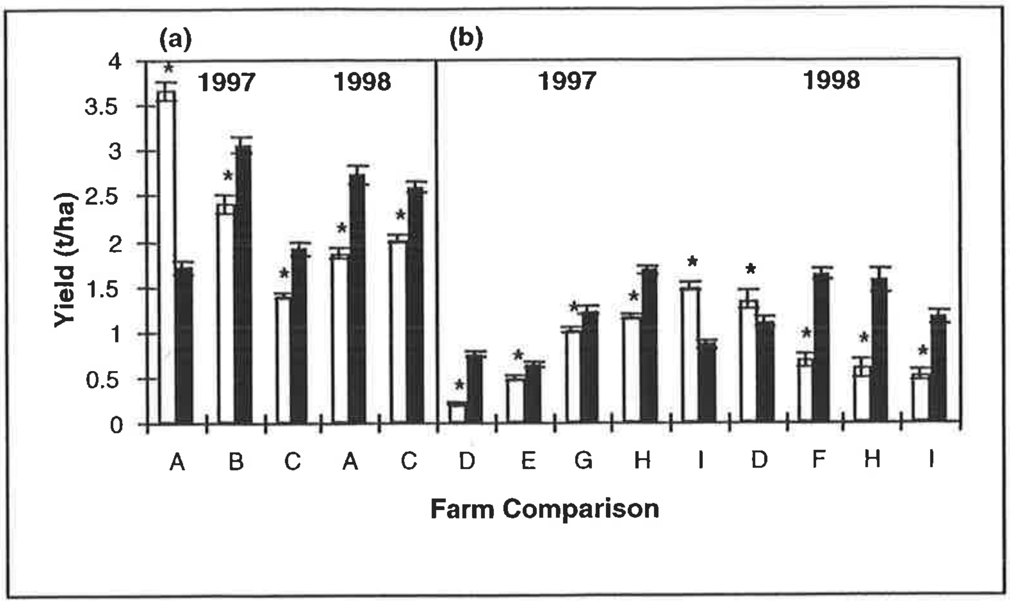


Figure 3.8 Grain yield of wheat (average of selected varieties) grown under organic (☐) and conventional (■) systems in high **(a)** and low **(b)** rainfall areas in 1997 and 1998. The asterisk indicates that the organic system is significantly different to the conventional system (P<0.05) in that comparison.

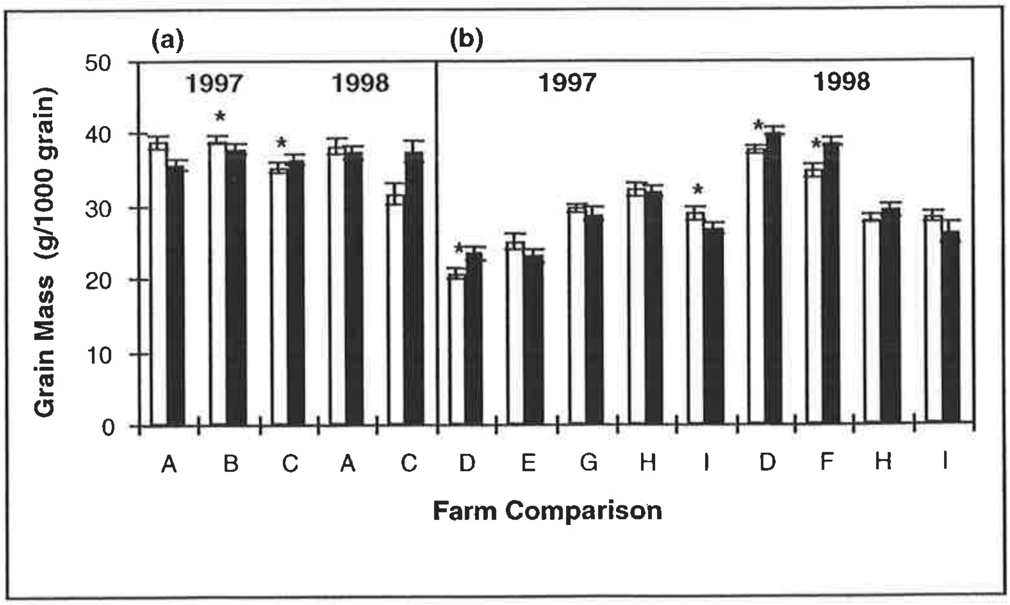


Figure 3.9 Thousand-grain weight of wheat (average of selected varieties) grown under organic (☐) and conventional (■) systems in high **(a)** and low **(b)** rainfall areas in 1997 and 1998. The asterisk indicates that the organic system is significantly different to the conventional system (P<0.05) in that comparison.

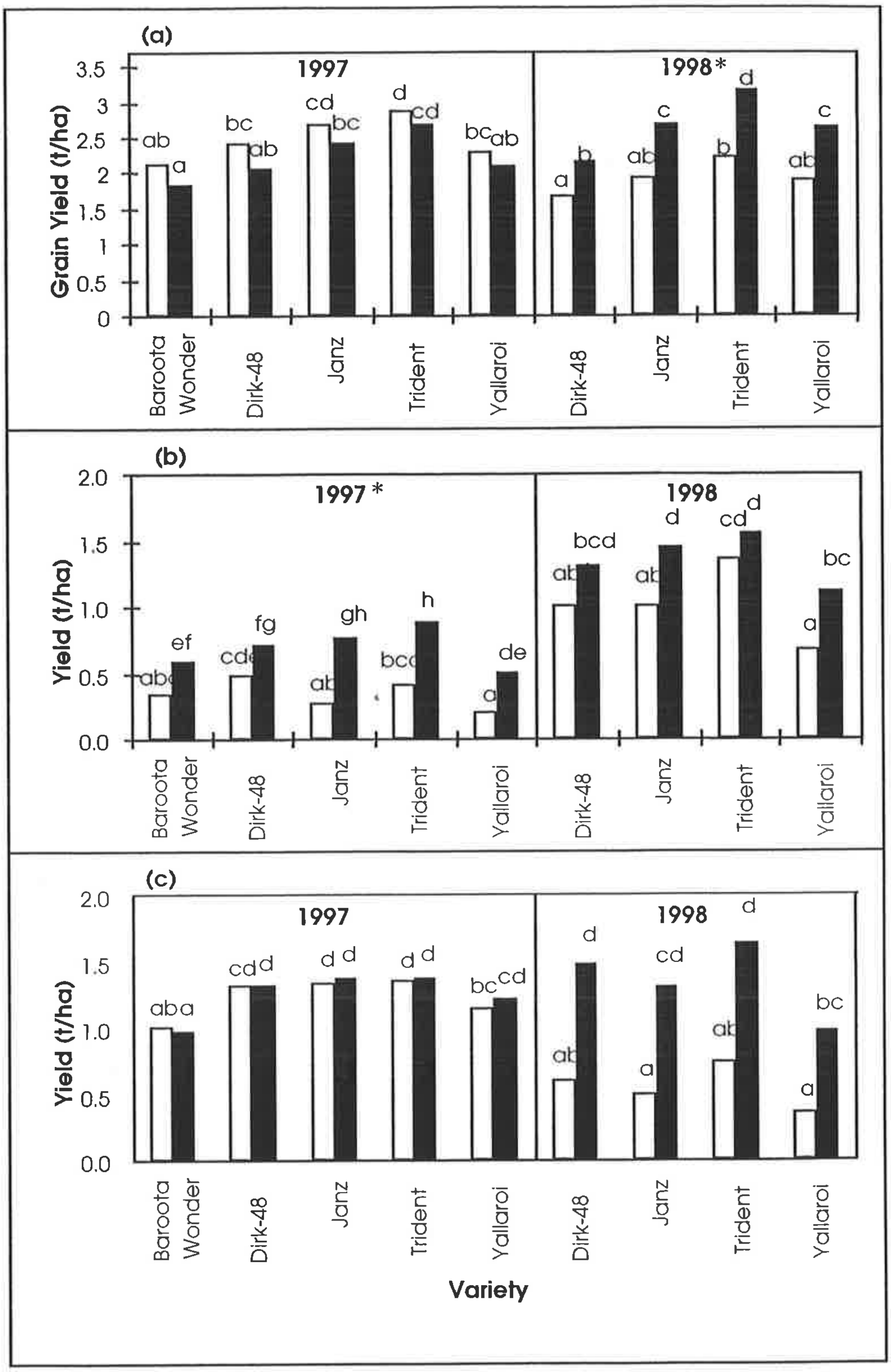


Figure 3.10 Grain yield (average of all farming system comparisons) of selected wheat varieties grown in organic (☐) and conventional (■) systems in high (**a**) and low (**b** and **c**) rainfall areas (Wolseley, Nadda and Wudinna respectively) in 1997 and 1998. Columns within the same season and with the same letter above are not significantly different (P>0.05). Asterisks indicate a significant variety by system interaction.

3.4.3 Grain Yield

The grain yield of organically grown wheat was lower than the conventional for most comparisons in the current study (Figure 3.8). Many previous comparisons have also reported lower yields from organically grown wheat (Lockeretz *et al.*, 1981; Stanhill, 1990; Dumaresq, 1992; Deria *et al.*, 1996; Derrick, 1996; Entz *et al.*, 1998). The degree of yield reduction in the high rainfall organic systems of the current study was similar to that reported in the majority of the comparisons in the literature (most organic systems yielding 10 to 30 percent lower than conventional systems). However, there were also examples in the literature of organic systems yielding as poorly, in comparison to conventional systems, as some of the low rainfall organic systems in the current study (Lockeretz *et al.*, 1981; Derrick, 1996).

It has been shown that in Australia, where wheat yields are often limited by rainfall, organic systems tend to yield higher, relative to conventional systems, in years when the conventional systems produce low yields due to low rainfall and other stresses (Wynen, 1994). Based on this report, it could be anticipated that the low rainfall, rather than the high rainfall, organic systems would produce yields much closer to those of conventional systems. However, this was not so in the current study. Four out of the seven low rainfall organic systems with yields lower than the conventional were substantially below the 21 to 31 percent yield reduction (compared to conventional) obtained in the high rainfall organic systems (Figure 3.8). The high rainfall organic systems were also much more stable in their variation from the conventional systems, regardless of season and site, than the low rainfall organic systems.

Furthermore, it cannot be concluded that the variation in the yield of the organic systems (expressed as a percentage of the yield of the conventional systems) is related to the rainfall in the growing season (Figure 3.11). In contrast with the findings of Wynen (1994), this points to there being factors that are more important than growing season rainfall that help determine the comparative performance of the organic systems. Possibilities include the number of sunlight hours, the timing of rainfall and stresses such as frost and heat during critical stages of physiological development. These could realistically make a difference to the comparative yield of the organic systems, particularly if plant development in the organic system is delayed due to late sowing or nutrient deficiencies.

The discrepancy with the findings of Wynen (1994) may also have been due to the weediness, particularly with broadleaf weeds, of organic systems (because of no herbicide application) compared with most of the conventional systems (Table 3.6). Under low rainfall conditions, weed competition for moisture can be highly detrimental to the growth and development of a crop, especially if the weeds are more developmentally advanced. This is usually addressed by organic farmers through pre-sowing cultivation and the use of wide tines in the sowing pass, but was not possible in the experimental plots due to the narrow tines on the plot seeder.

Because wheat grown in organic systems often yields less than in comparable conventional systems, the economic sustainability of organic farming systems becomes an issue of importance. Inputs are usually less, however, in organic systems and therefore the cost of production is also less. In addition to this, there are currently price premiums available in Australia for organically grown wheat.

Although organic systems commonly yield less than conventional systems, there have also been examples reported of organic systems yielding more (Stanhill, 1990; Dumaresq, 1992). The comparisons within the current study for which the organic system yielded greater than the neighbouring conventional system, therefore, have parallels in the literature.

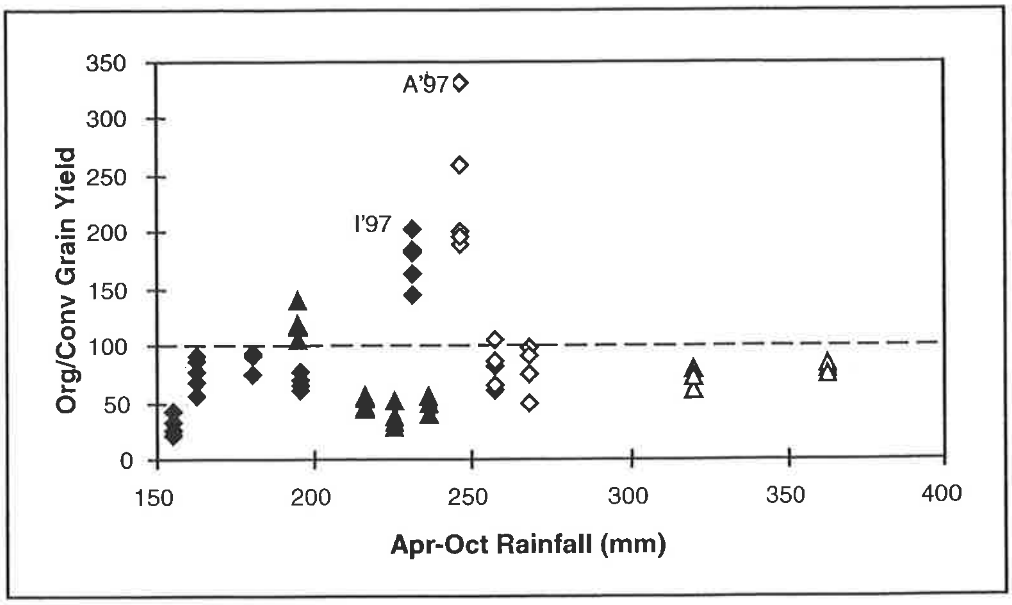


Figure 3.11 Grain yield of the organic systems (expressed as a percentage of the conventional yield) plotted against the growing season rainfall. A value for each variety has been included to indicate variability. The open symbols are the high rainfall comparisons and the closed symbols are the low rainfall comparisons. Diamonds represent 1997 comparisons and triangles 1998 comparisons.

Table 3.6 The average number of grass and broadleaf (B/leaf) weeds per m^2 in organic (Org) and conventional (Conv) comparisons in 1997 and 1998.

Comparison			1997		1998	
			Grass Weeds	**B/leaf Weeds**	**Grass Weeds**	**B/leaf Weeds**
Wolseley	A	Org	16	28	9**	10**
		Conv	64	20*	17	6*
	B	Org	2	168	NE	NE
		Conv	4	50	NE	NE
	C	Org	96	174	24	38
		Conv	4	26*	38	19*
Nadda	D	Org	328	30	2	54
		Conv	24	32*	49	27*
	E	Org	54	58	NE	NE
		Conv	100	126*	NE	NE
	F	Org	NE	NE	46	31
		Conv	NE	NE	42	2
Wudinna	G	Org	6	254	NE	NE
		Conv	40	26	NE	NE
	H	Org	390	8	258	292
		Conv	298	2	71	1
	I	Org	20	2	870	35
		Conv	8	62*	123	8

NE indicates no experiment in that season.

*Herbicide was applied mid-season to control these weeds.

**Weed counts after the use of a mechanical weeder.

High yields in an organic system, relative to a conventional system, may be due to favourable environmental conditions (or unfavourable conditions for the conventional system), to good management decisions in that year or, most likely, to a situation where management decisions happened by chance to combine well with the environmental conditions for that year. An example of this would be the difference in sowing date normally expected between the systems. A later sown organic crop may be sufficiently delayed in development to avoid frost that damages the earlier sown conventional crop.

In the current study, the organic system from comparison A in 1997 more than doubled the yield of the conventional system (Figure 3.8 (a)) and for this reason calls for further attention. The doubling of the conventional yield appears to be a combination of the organic system yielding particularly well and the conventional system yielding fairly low in that site pair. The higher yield by the organic system is obviously not due to perfected management by the organic farmer from comparison A because in 1998, the organic system at comparison A yielded much lower than in 1997 and considerably lower than the conventional system also. The high yield of the organic system in 1997 was due mostly to greater grain numbers rather than heavier grain (although there was a non-significant trend towards heavier grain also).

The rainfall at Wolseley during the growing season was greater in 1998 than in 1997 (Figure 3.3) and, therefore, it would be expected based on the comparison by Wynen (1994), that relative to the conventional, the organic system would yield higher in 1997 (the drier year) than it would in 1998. However, all Wolseley comparisons received similar rainfall but comparison A is the only one in which the organic system yielded greater than the conventional system, and in fact recorded the highest yield in this study, conventional systems included. It can be concluded therefore, that climate alone does not account for the high yield of the organic system from comparison A in 1997 but that management factors are also involved.

There are two management factors that are implicated in the high yield of the organic system in 1997. The first is the addition of composted mineral fertiliser to the organic system of comparison A (1997) and not to any other site. This fertiliser (and guano, also applied only to the organic system of comparison A (1997)) is the only amendment used in the organic systems that contains nitrogen. This added nitrogen may have contributed to the high yield of the organic system.

The second possible factor is that the paddock used was fallow in the previous year, rather than pasture or crop. There was only one other instance of a site being fallow the previous year and that was the conventional system from comparison B (1997). Interestingly, this was the only other site to have an average yield above 3 t/ha. The importance of fallow for retaining pre-season moisture in the soil is contested by some

(Kohn *et al.*, 1966) but 1997 was much drier than 1998 during the early growing season (June and July) and fallowed sites may have benefited from conserved moisture. However, the organic system from comparison A in 1997 had a lower biomass at late tillering than the conventional system, indicating that if retained moisture was important, it did not translate to increased growth in the organic system during the dry months. It is possible though, that the lower available soil phosphorus (Table 3.4) in the organic system limited early vegetative growth but that the moisture conserved by the fallow was effective later in the season in increasing the grain production. This may have been facilitated through reducing the competition with weeds for moisture. A well managed fallow also has the advantage of reducing the weed load in the following year through a decreased seed-bank.

There are a number of reasons why organic systems usually yield less than conventional systems:

(i) Varieties chosen by organic farmers (often for marketing reasons) often have a lower yield potential in conventional systems than the varieties grown by conventional farmers in the same region. This was the case in the study by Derrick (1996) in New South Wales. However, in the current study, this variable was not confounding the data because the same range of varieties were sown in both the organic and conventional systems. Even amongst the varieties chosen it can be seen that there is a range in yields, in both the organic and conventional systems (Figure 3.10), although in most cases there was not a significant interaction between variety and system.

(ii) Many organic farmers sow later than their conventional counterparts in order to control the weeds prior to sowing. This is thought to be an important factor contributing to the reduced yield of organically grown cereals because delay in sowing date within conventional systems has been shown to reduce grain yield by up to 250 kg/ha/week (French and Schultz, 1984a). This factor, however, has been removed from the current study, with both organic and conventional systems within each site pair being sown on the same day.

Using the same sowing date for both farming systems, however, resulted in a greater weed burden within the organic systems. The presence of weeds, unchecked by herbicide, within organic systems is another important factor contributing to yield loss. Weed infestation on conventional farms has been shown to decrease yield by reducing water use efficiency and by competing for light and nutrients (French and Schultz, 1984b). A yield reduction of 36 g of grain for each additional 100 g of weed biomass was calculated by Kohn *et al.* (1966). In the current study the infestation of weeds was certainly more severe, and mostly unchecked, on the organic farms (Table 3.6) and could account for some of the yield reduction. Also, the conventional system in comparison A (1997) had a high infestation of herbicide resistant rye grass and this possibly contributed to the reduced conventional yield in that comparison. Derrick (1996) showed that weed biomass was always greater in the organic system than the conventional in each of three seasons, but even so, that the total above ground biomass including weeds was greater in the conventional system. This indicates that there are other factors contributing to lower yield in the organic system.

(iii) Perhaps the most important factor contributing to yield loss in organic systems is nutrient deficiencies due to nutrient removal in grain and the failure to apply soluble fertilisers as nutrient replacement. This factor, particularly in relation to phosphorus deficiency, will be discussed in Chapter 4.

The thousand-grain weight was not consistently different between wheat grown in the organic and conventional farming systems in the current study (Figure 3.9). However, Derrick (1996) found organically grown grain to have a consistently lower thousand-grain weight than conventionally grown grain. This result may have been due to the comparison in that study of different wheat varieties in each farming system. This was not a factor in the current study.

The observation that Trident (zinc efficient) yielded consistently high and Yallaroi (zinc inefficient) mostly low in both systems and in high and low rainfall areas of South Australia (Figure 3.10), may point to the presence of zinc deficiency in the Wolseley, Nadda and Wudinna areas. More importantly, it indicates that neither of these varieties was better adapted to the conditions in the organic systems than the conventional systems. In fact, none of the varieties included in this study showed a consistent grain production adaptation to either of the farming systems. This contradicts the hypothesis, suggested at the beginning of the chapter, that the older varieties may be better adapted to organic systems than the more recently released varieties. However, it is important to consider that only two out of many wheat varieties released early in the twentieth century were included in this study.

German winter wheat varieties that were successful early in the twentieth century were found to yield less in low external input (for example, organic) systems than modern varieties (Stoppler *et al.*, 1990). These researchers found that one of the oldest varieties showed enhanced root growth during grain fill, compared with the modern varieties. They hypothesised that this could result in a reallocation of resources during grain development with the consequence of reduced grain yield.

Baroota Wonder produced very low yields or yields comparable to Dirk-48 in all experiments (Figure 3.10) and for this reason, only Dirk-48 was carried over into the 1998 experiments. Dirk-48 generally produced yields in the mid-range compared to the other varieties in both the organic and conventional systems (Figure 3.10).

3.5 Summary

- The first aim of this study was to compare the growth and yield of wheat grown in organic and conventional farming systems under South Australian conditions.

Like other areas in Australia and overseas, it was found that wheat grown in organic systems in South Australia generally yielded lower than in conventional systems in both biomass and grain yield. However, there were several exceptions in which the organic system yielded greater than the conventional system.

- The second aim was to assess the importance of rainfall on the growth and yield of organic systems relative to conventional systems.

The grain yield of the organic systems in the high rainfall area of South Australia was mostly between 70 and 80 percent of the conventional yields. In the low rainfall areas, the grain yield of the organic systems (as a proportion of the conventional yield) was much more variable and as low as 28 percent of the conventional system in one comparison. However, this variability was not closely associated with variation in the seasonal rainfall, as was suggested by Wynen (1994).

- The third aim was to investigate the influence of selected wheat varieties, including old wheat varieties, on growth and yield of organic systems.

Varieties of wheat that had been developed before widespread fertiliser and biocide use did not appear to be better adapted to the organic system than the conventional system, either in terms of biomass production or grain yield. Similarly, the modern varieties did not have adaptive advantage in either farming system.

Chapter 4

Comparison of Organic and Conventional Farming Systems: Plant Nutrition and Grain Composition

4.1 Introduction

This study was designed to compare wheat grown in organic and conventional farming systems in order to identify any existing differences in mineral and amino acid composition of the grain.

It has been shown in Chapter 3 that most of the organic systems produced less biomass and grain yield than comparable conventional systems. These differences in growth and yield have the potential to alter the nutritional status of the plants and to concentrate the nutrient composition of the grain produced.

In a comprehensive sustainability study in New South Wales by Derrick (1996), it has been shown that phosphorus fertiliser regimes in organic and conventional systems result in lower phosphorus concentrations in the grain of wheat grown in organic systems. The management of the organic systems in New South Wales is similar in many respects to that of organic systems in South Australia. However, the environmental conditions are different. It is important, therefore, to determine how applicable this observation is, particularly in the low rainfall conditions of much of the South Australian wheat belt.

The concentrations of other nutrients in the grain could also be expected to differ, either as an interaction with phosphorus or through an independent mechanism caused by other management practices within the organic system, particularly as a concentration or dilution effect in relation to grain yield (refer to Chapter 2, section 2.4.2).

The aims of this part of the field study were, therefore, fourfold:

- To assess the nutrient status of wheat crops grown in organic systems in both high and low rainfall environments within South Australia,
- To compare the grain phosphorus levels of organic and conventional systems under a range of management and environmental conditions,

- To identify nutrients other than phosphorus which have consistently different levels in organically and conventionally grown grain and
- To investigate the effect of organic and conventional farming systems on protein concentration and amino acid composition of grain.

4.2 Methods

The paired comparisons of organic and conventional systems described in Chapter 3 were used for the nutritional investigation.

4.2.1 Tissue and Grain Analysis

Plant tissue (whole shoot) was collected at late tillering in 1997 and at late tillering, anthesis and maturity (prior to harvest) in 1998 as detailed in Chapter 3. At maturity, the heads were removed from the remaining whole tops and threshed. The grain and straw were analysed separately.

All tissue samples were oven dried and weighed and three (1997) or four (1998) replicates were ground and analysed. Nitric acid digestion was modified from procedures detailed by Zarcinas *et al.* (1987) (details in Appendix 3). The samples were analysed for iron, manganese, copper, zinc, calcium, magnesium, sodium, potassium, phosphorus and sulphur concentrations using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES). Critical levels of nutrients in the tissue at late tillering were determined from papers cited by Reuter *et al.* (1997). Where no critical values were available for whole shoots at late tillering, critical levels were either estimated by interpolation of deficient and adequate values (from Reuter *et al.*, 1997) for that nutrient in whole shoots at late tillering or by using the critical value in whole shoots (from Reuter *et al.*, 1997) for that nutrient at the closest developmental stage to late tillering. Where multiple critical values were reported by Reuter *et al.* (1997), Australian values were used. The total uptake by the plants (g of nutrient per ha) was estimated by multiplying the nutrient concentration of the tissue by the biomass production.

For the grain samples (the assumption was made that the small plot harvester delivered an evenly mixed sample and thus sophisticated sub-sampling was unnecessary), four replicates were digested using nitric and perchloric acids and analysed for elemental composition using ICPAES (details in Appendix 3). Nutrient removal from the farm due to grain harvest was estimated by multiplying the nutrient concentration of the grain by the grain yield.

Of the grain samples, two replicates of two varieties (Janz and Trident) from comparisons A and C in 1997 and 1998, and two replicates of Janz from comparisons H and I in 1997 and D and F in 1998, were finely ground, extracted with hydrochloric acid (method of Lehrfeld (1994)) and analysed for phytate using High Performance Liquid Chromatography (HPLC) (details in Appendix 3).

The same finely ground grain samples used for phytate analysis were analysed for amino acid concentrations. The samples were hydrolysed (based on Barkholt and Jensen, 1989) and analysed using the HPLC method of Blankenship *et al.* (1989) (details in Appendix 3).

4.2.2 VAM Screening

Roots of four plants were collected from a single replicate of each of two varieties (Dirk-48 and Janz) within each 1998 comparison at early tillering (around developmental stage 22 (Zadoks *et al.*, 1974). This was achieved by using a trowel to remove the plants with roots intact to a depth of 15 cm. These samples were used for Vesicular Arbuscular Mycorrhizal fungi (VAM) screening. These roots were stored in 70% ethanol after washing (four plants bulked together) and later cleared in 10% potassium hydroxide for five days, rinsed in 1N hydrochloric acid,stained for one hour in analine blue, rinsed and stored in 50% glycerol (method modified from Phillips and Hayman, 1970). It is to be noted that phenol was omitted from the reagents. Percentage VAM colonisation was determined using the gridline intersect method (Giovannetti and Mosse, 1980).

4.2.3 Statistical Analysis

All data sets were analysed using GENSTAT 5 as detailed in Chapter 3. Data were analysed separately for each comparison of organic and conventional systems in order to identify anomalous comparisons. Although the variety treatments were accounted for in the ANOVA, data presented in this chapter was averaged across varieties because the effect of the farming system was the primary focus. Averaging across the varieties was valid because out of 96 ANOVA, there were only seven ANOVA with a significant system by variety interaction. None of these interactions were consistent with each other and five of them did not involve an inversion of the system effect. The two cases where a system inversion trend was present in one variety (grain Mn concentration at Nadda in 1997 and grain zinc concentration at Nadda in 1998), the result was not consistent with data from other locations or seasons.

The nutrient concentration data for plant tissue at late tillering and for grain were analysed with all sites and both seasons combined to achieve greater statistical power.

This will henceforth be referred to as the mega-analysis and also included a stratum testing the effect of high (Wolseley) or low (Wudinna and Nadda) rainfall.

The root infection (VAM) data were based on single replicates of each system and variety within each comparison. Hence these were analysed by treating the comparisons as replicates of the systems.

4.3 Results

4.3.1. Overview of Elemental Analyses

The results of the mega-analysis, showing the effect of farming system (organic or conventional) and the interaction of farming system with the rainfall environment on grain and tissue elemental concentrations, are displayed in Table 4.1.

There was no overall significant effect of farming system on concentrations of iron, calcium or sulphur in tissue or grain and no overall significant effect on the concentration of sodium in the grain (Table 4.1 and Appendix 4). The conventionally grown wheat had significantly greater sodium in plant tissue at late tillering than organically grown wheat.

For manganese, magnesium, potassium and phosphorus, the conventionally grown wheat had greater concentrations than the organically grown wheat in both plant tissue at late tillering and in the grain (Table 4.1). The organically grown wheat had greater concentrations of copper and zinc (Table 4.1). All these effects were highly significant.

Because of the differences between the organic and conventional farming systems in phosphorus, magnesium, potassium, manganese, copper and zinc concentrations, these elements will be investigated more closely in the following sections.

The differences between farming systems in respect to manganese, magnesium and phosphorus concentrations in the plant tissue were much greater in the low rainfall environments than the high rainfall environment (Table 4.1). Furthermore, the manganese concentrations in plant tissue from both conventional and organic farming systems in the low rainfall environments (averaged 66.5 and 44.7 mg/kg respectively) were greater than the concentrations in high rainfall conventional and organic systems (averaged 40.0 and 36.8 mg/kg respectively). In contrast, the grain zinc concentrations from both conventional and organic farming systems in the high rainfall environment (averaged 21.1 and 27.3 mg/kg respectively) were greater than both farming systems in the low rainfall environments (averaged 18.5 and 20.7 mg/kg respectively). Also, there was a greater difference in grain zinc concentrations between farming systems in the high rainfall environment than the low rainfall environments (Table 4.1).

Table 4.1 A summary of the statistical results in the mega-analysis (14 sites over 2 seasons) of elemental composition (mg/kg) of tissue (whole tops) at late tillering and grain in comparisons between organic (Org) and conventional (Conv) farming systems.

Element	**Tissue (n=192)**	**Sys.Rainfall# Interaction**	**Grain (n=224)**	**Sys.Rainfall Interaction**
Fe	NS	NS	NS	NS
Mn	Conv > Org ***	Effect greater for LR ***	Conv > Org ***	NS
Cu	Org > Conv **	NS	Org > Conv ***	NS
Zn	Org > Conv ***	NS	Org > Conv ***	Effect greater for HR **
Ca	NS	NS	NS	NS
Mg	Conv > Org ***	Effect greater for LR ***	Conv > Org ***	NS
Na	Conv > Org *	NS	NS	NS
K	Conv > Org **	NS	Conv > Org ***	NS
P	Conv > Org ***	Effect greater for LR *	Conv > Org ***	NS
S	NS	Effect opposite in HR & LR **	NS	NS

#The interaction of farming system with high (HR) or low (LR) rainfall environment is indicated by the Sys.Rainfall interaction term. *0.01<P<0.05 **0.001<P<0.01 ***P<0.001 NS indicates no significant effect.

The nutrient uptake per hectare by plants in the conventional system at late tillering was greater than or equal to those of the organic system for all nutrients investigated within all comparisons in the high rainfall environment (Table 4.2 and Appendix 5). It was also true for all comparisons in the low rainfall environments except for comparison I (1997), the comparison in which the conventional system showed abnormally low early growth (see Chapter 3, section 3.3.2) so that the organic system had produced greater biomass by late tillering.

Similarly, the grain nutrient content per hectare in the conventional system was greater than or equal to the organic system in all cases except for the three comparisons (A, 1997; I, 1997 and D, 1998) for which the organic system produced higher grain yields than the conventional system (Table 4.2 and Appendix 6).

Table 4.2 The number of comparisons of organic and conventional farming systems in which the nutrient uptake (g/ha) into tissue at (late tillering) and the grain (at maturity) was significantly greater in conventional (Conv) or organic (Org) systems from high (HR) and low (LR) rainfall environments.

Element Uptake (g/ha)	Tissue						Grain					
	Conv > Org		NS		Org > Conv		Conv > Org		NS		Org > Conv	
	HR	LR	HR	LR	HR	LR*	HR	LR	HR	LR	HR*	LR*
Fe	1	6	4	2	0	1	3	6	1	2	1	1
Mn	3	7	2	2	0	0	3	5	1	3	1	1
Cu	2	5	3	4	0	0	1	5	3	2	1	2
Zn	2	6	2	2	0	1	0	5	4	2	1	2
Ca	4	6	1	3	0	0	0	6	4	2	1	1
Mg	4	6	1	3	0	0	3	6	1	2	1	1
Na	2	4	3	5	0	0	0	2	4	5	1	2
K	3	6	2	2	0	1	2	6	2	2	1	1
P	4	7	1	2	0	0	3	6	1	3	1	0
S	4	6	1	3	0	0	2	6	2	2	1	1

NS indicates no significant difference between farming systems. *All comparisons in these columns are ones where the organic system yielded greater than the conventional system ie. HR: comparison A, 1997; LR: comparisons I, 1997 and D, 1998.

4.3.2 Phosphorus

Phosphorus was added as fertiliser to all conventional systems in both seasons. It was also added to all of the organic systems at Wolseley, the high rainfall area (except in comparison B, 1997). However, the forms of phosphorus used within the organic systems were less available than those used on the conventional systems (Tables 3.1, 3.2 and Appendix 1).

The conventionally grown wheat had consistently higher phosphorus concentrations at late tillering than the wheat from the organic systems (Figure 4.1). This was true in both the high and low rainfall environments. It was also consistent throughout the season (late tillering, anthesis and in the straw at maturity) and was mirrored in the grain (Figures 4.2 and 4.3).

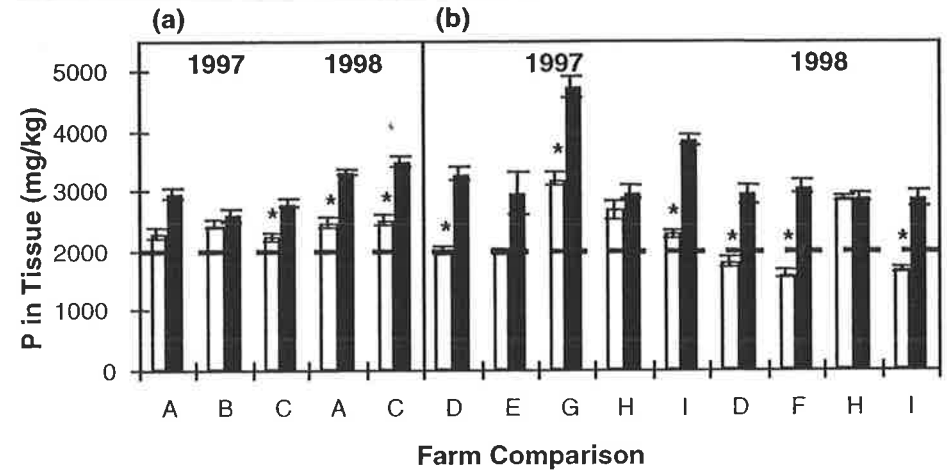


Figure 4.1 The phosphorus concentration at late tillering of the whole tops of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$). The broken line is the critical phosphorus level in whole tops (Reuter *et al.*, 1997).

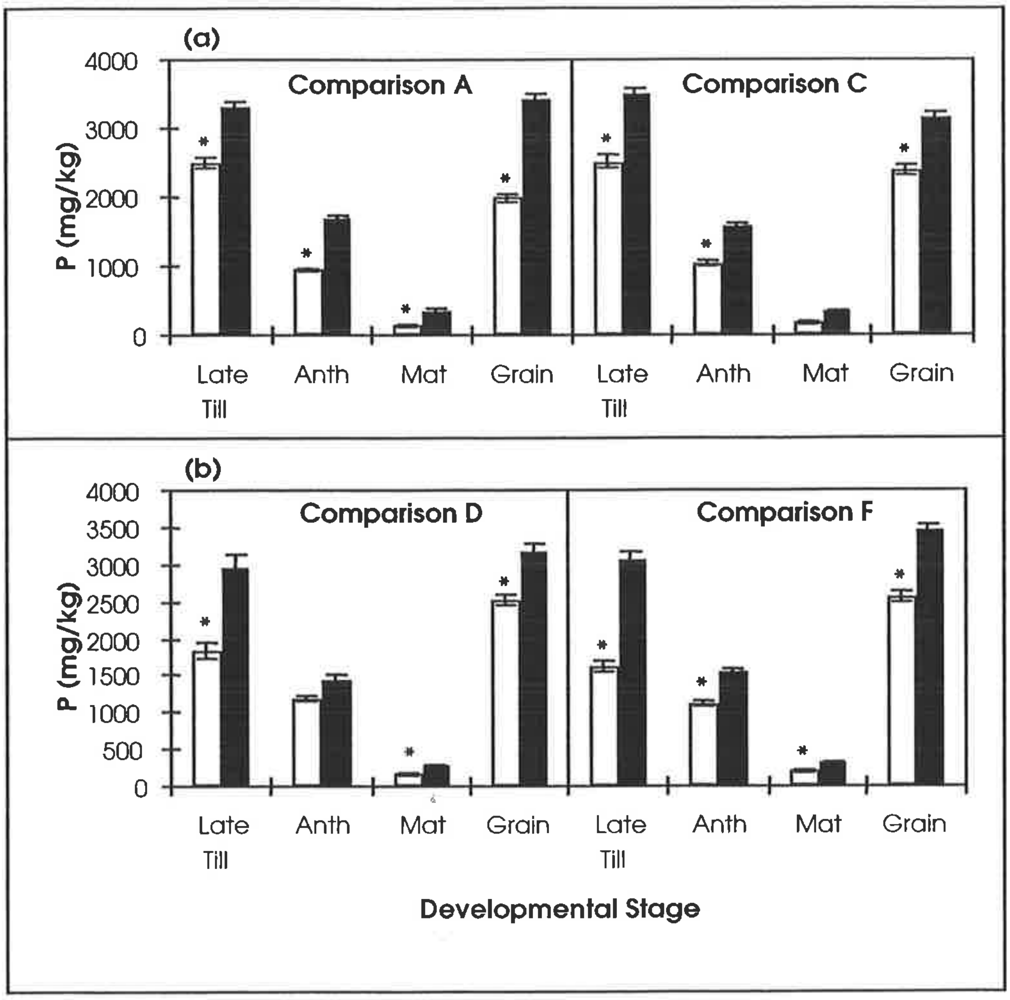


Figure 4.2 The phosphorus concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of wheat plants grown in organic (☐) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$).

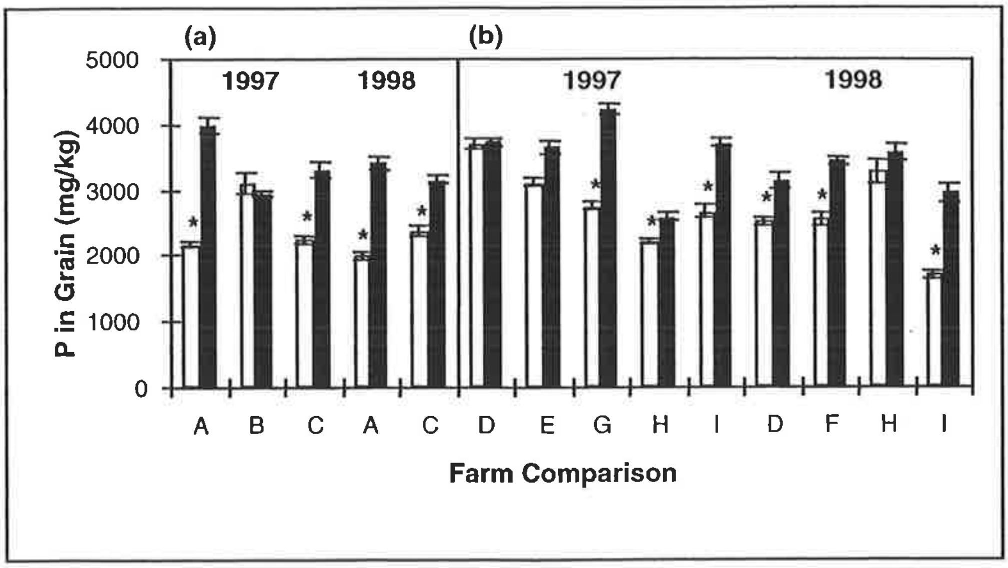


Figure 4.3 The phosphorus concentration in the grain of wheat grown organically (□) and conventionally (■) in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

All of the conventional systems were well above the critical phosphorus level, indicating phosphorus sufficiency, at late tillering (Figure 4.1). However, a number of the organic systems, particularly in the low rainfall areas, were below that critical level.

The concentration of phytate in the grain followed the same pattern as phosphorus, with wheat from the conventional system having a greater phytate concentration than the organically grown wheat (Figure 4.4). However, there were similar proportions of phytate phosphorus and non-phytate phosphorus in the grain from both farming systems (Figure 4.5).

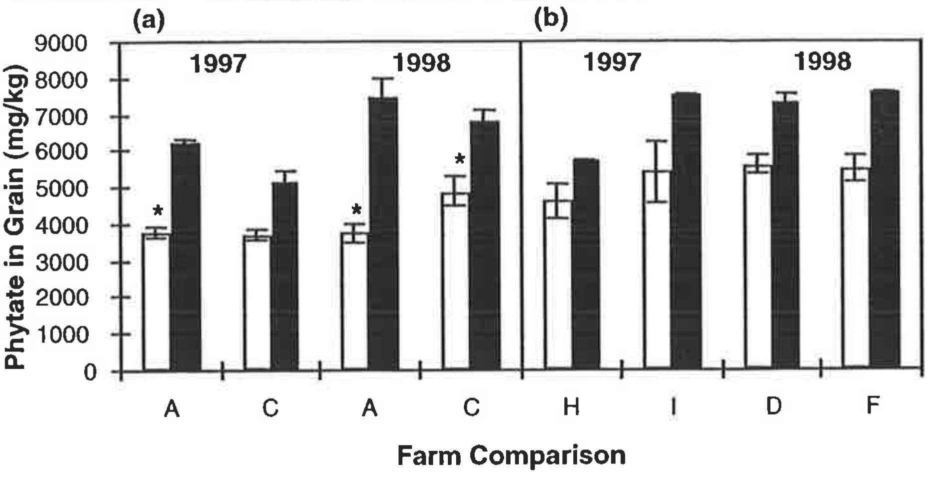


Figure 4.4 The phytate concentration in the grain of organically (□) and conventionally (■) grown wheat from high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

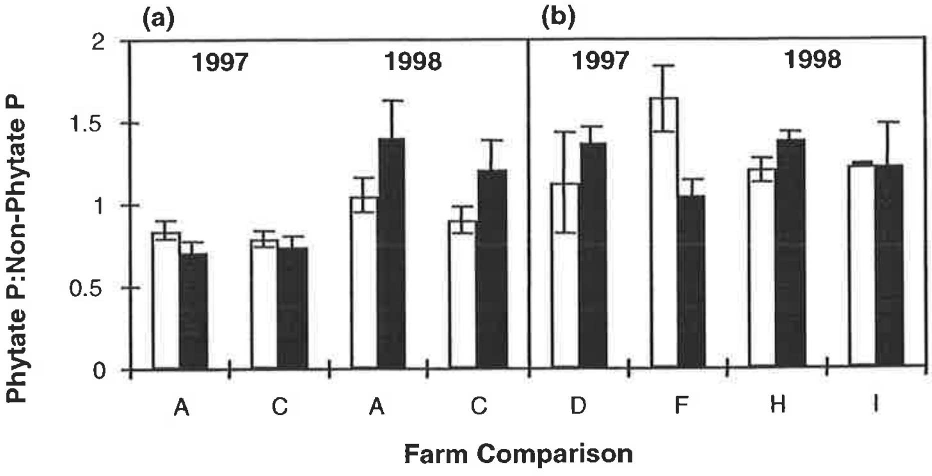


Figure 4.5 The ratio of phytate phosphorus to non-phytate phosphorus in organically (□) and conventionally (■) grown wheat grain from high **(a)** and low **(b)** rainfall environments. The organic system was not significantly different to the conventional system in any comparison.

4.3.3 Magnesium

Magnesium was not added as fertiliser by conventional farmers, however it was present (0.4-3%) in fertilisers added to the organic system, resulting in additions of 0.6-6 kg of magnesium per hectare in all comparisons except comparisons B and G in 1997.

The magnesium level in the soil was greater in the organic systems compared with the conventional systems, but only in the low rainfall environments (Table 3.4 and Appendix 2).

In the low rainfall environments, the conventionally grown wheat had consistently greater magnesium concentrations at late tillering than the organically grown wheat. However, there was no clear difference in plant tissue magnesium concentration between the farming systems in the high rainfall environment (Figure 4.6). The magnesium concentration in the plants through the season was inconsistent (Figure 4.7) but in all comparisons, the magnesium concentration in the conventionally grown grain was greater than or equal to that from the organic system (Figures 4.7 and 4.8).

There were some comparisons, both organic and conventional systems, which were below the critical magnesium level at late tillering (Figure 4.6).

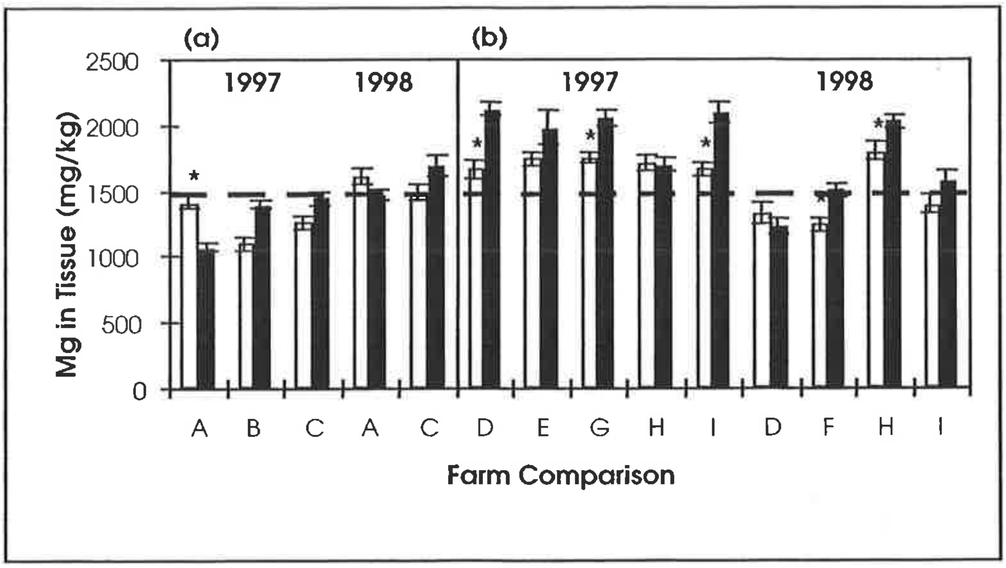


Figure 4.6 The magnesium concentration at late tillering of the whole tops of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05). The broken line is the critical magnesium level in whole tops (Melsted *et al.*, 1969 (cited by Reuter *et al.,* 1997)).

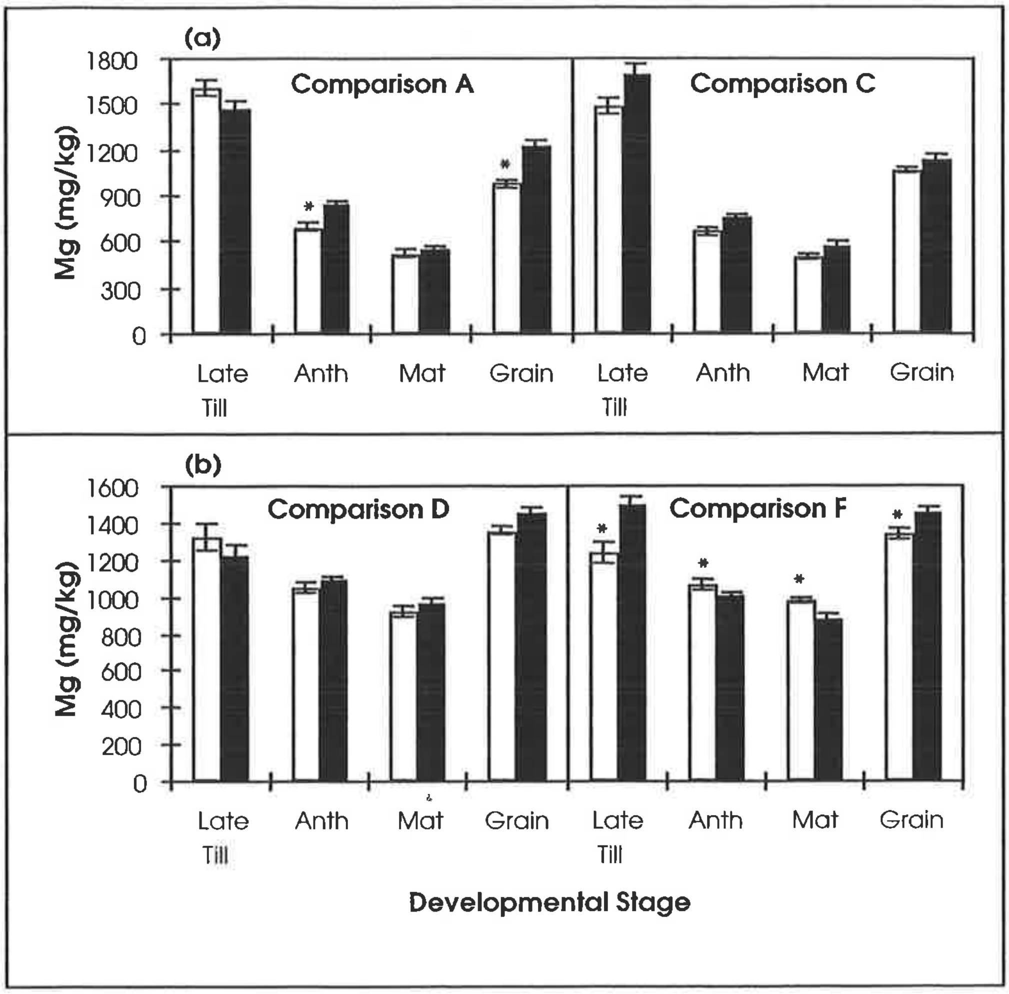


Figure 4.7 The magnesium concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$).

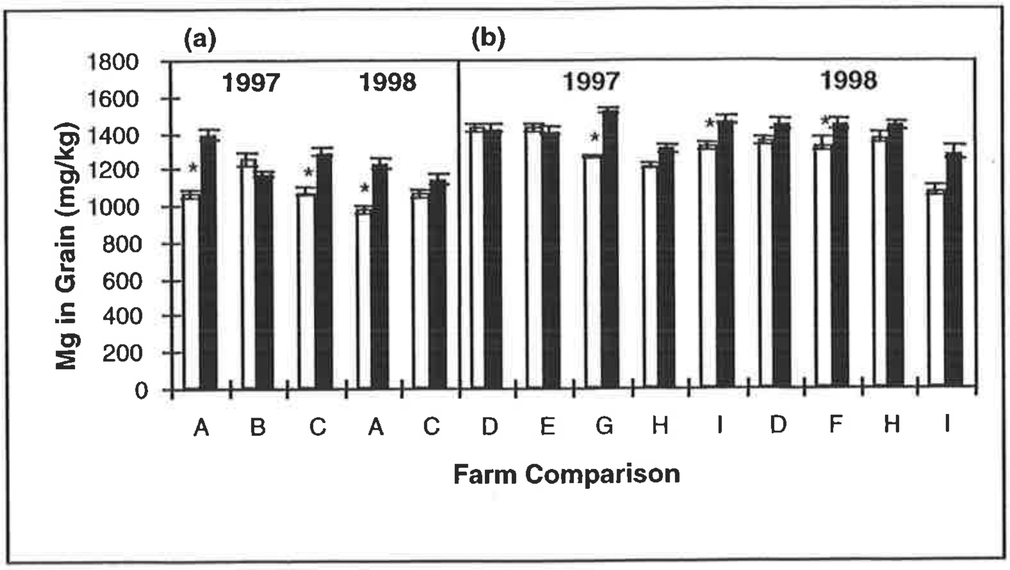


Figure 4.8 The magnesium concentration in the grain of wheat grown organically (☐) and conventionally (■) in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

4.3.4 Potassium

Like magnesium, potassium fertiliser was not added to any of the conventional systems but was present (0.25-2.5%) in products applied to the organic systems. This resulted in the addition of 0.08-2 kg of potassium per hectare in all comparisons except comparisons B and G in 1997.

At late tillering, the conventionally grown plants had greater or equal concentrations of potassium than the organically grown plants in most cases (Figure 4.9). Like magnesium, the comparative potassium concentrations through the season were somewhat inconsistent between the farming systems (Figure 4.10). However, the conventionally grown wheat had consistently greater potassium in the grain than the organically grown wheat (Figure 4.11).

The critical level of potassium in the plants at late tillering was amply exceeded by both farming systems in all comparisons (Figure 4.9).

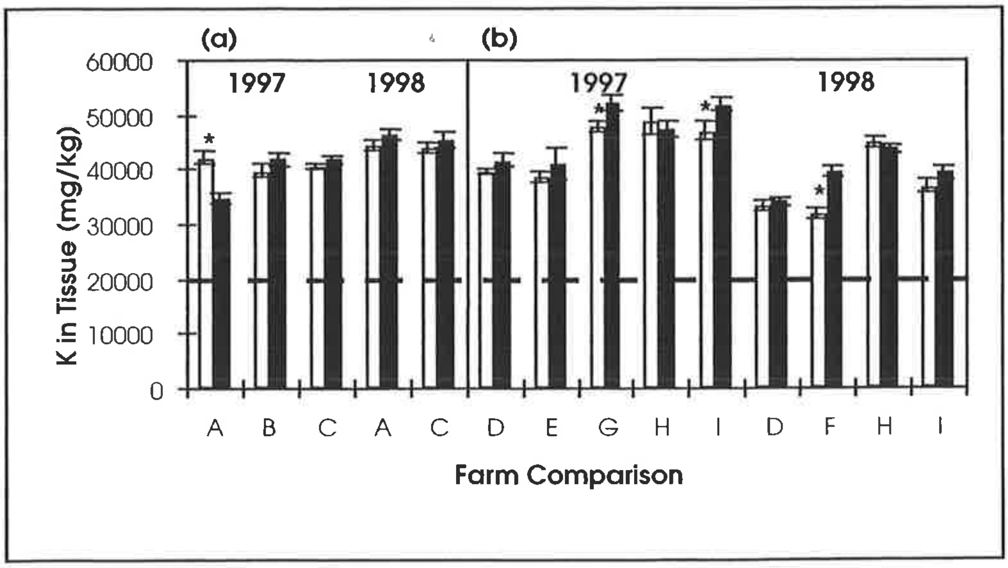


Figure 4.9 The potassium concentration at late tillering of the whole tops of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$). The broken line is the critical potassium level in whole tops (Cox, 1981 (cited by Reuter *et al.*, 1997)).

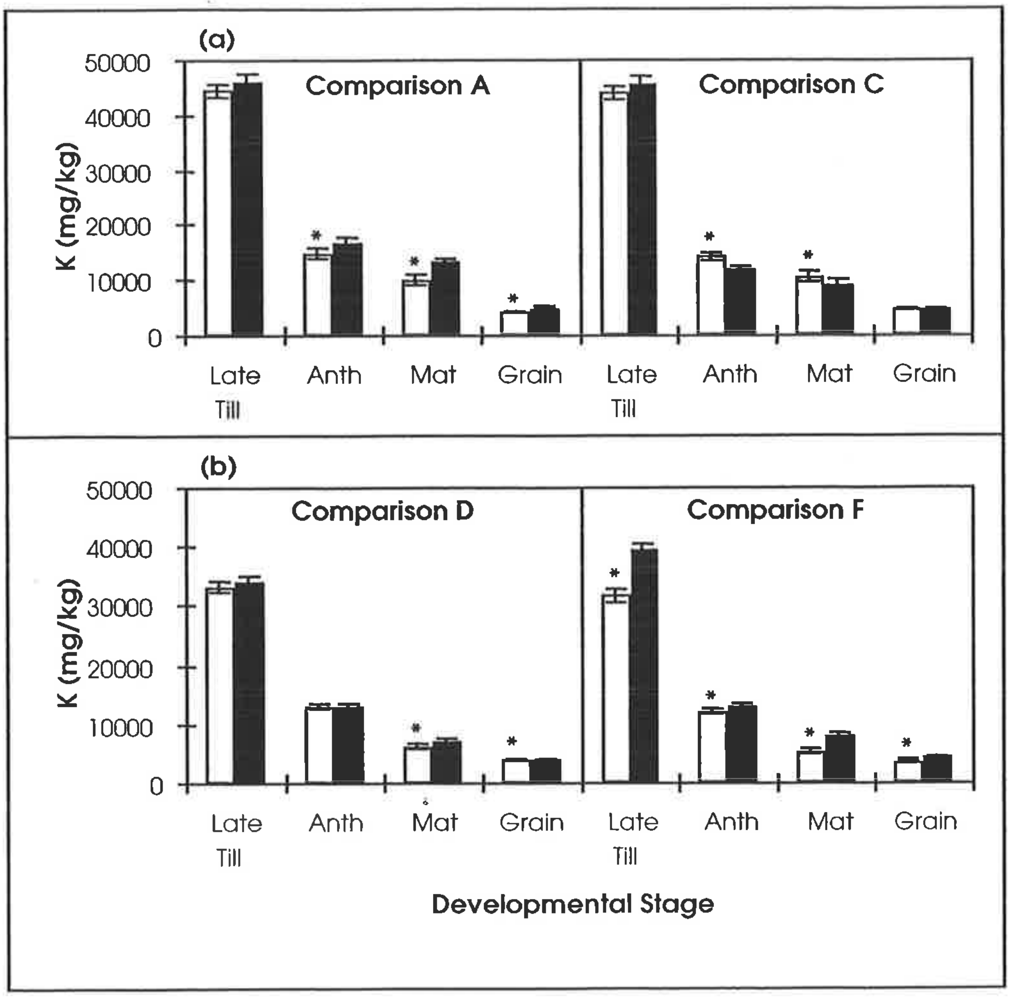


Figure 4.10 The potassium concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of wheat plants grown in organic (☐) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage (P<0.05).

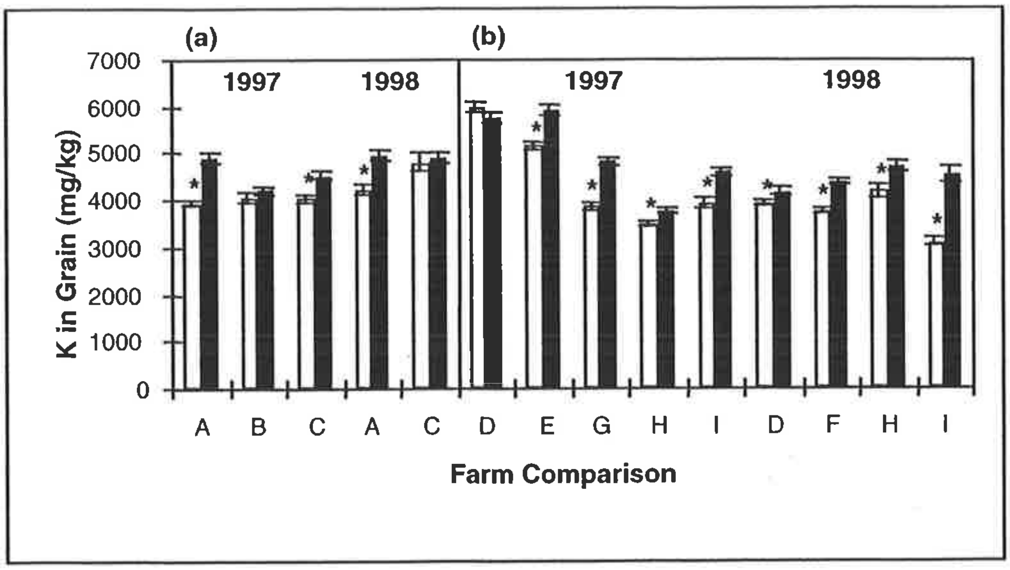


Figure 4.11 The potassium concentration in the grain of wheat grown organically (☐) and conventionally (■) in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

4.3.5 Manganese

Manganese chelate was applied as a foliar spray to both the organic and conventional systems of comparison C (1997) (Appendix 1). Manganese was not applied to any other of the conventional systems. However, it was present (levels ranging from 500-9500 mg/kg, values quoted in sales catalogues or determined by external laboratory analyses in the absence of a sales catalogue) in organic amendments applied to all organic systems except for those of comparisons B and G in 1997. This resulted in 15-300 g of manganese added per hectare to the organic systems and 1.7 kg per hectare added to the organic system in comparison A (1997).

In the low rainfall environment at late tillering, manganese concentrations in the conventionally grown plants were greater than or equal to the organically grown plants. In the high rainfall environment, however, there was less difference between the farming systems (Figure 4.12). The results at late tillering were consistent through the season (Figure 4.13) and reflected also in the grain manganese concentrations at maturity (Figure 4.14). The only exception was comparison H (1998) in which the organically grown grain had a significantly greater manganese concentration.

The manganese concentrations in the plant tissue at late tillering indicated that both the organic and conventional systems in all comparisons were replete in manganese (Figure 4.12).

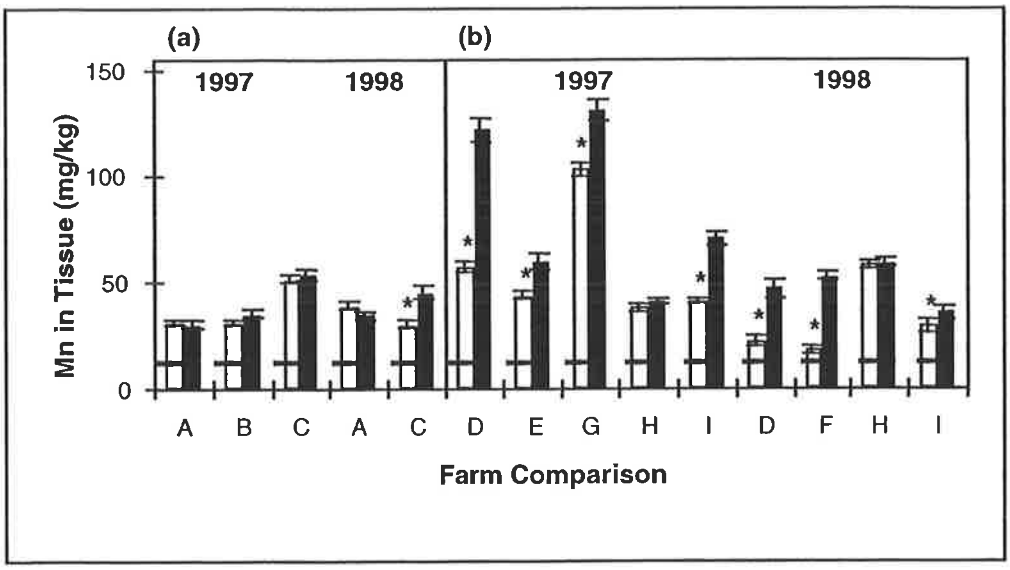


Figure 4.12 The manganese concentration at late tillering of the whole tops of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05). The broken line is the critical manganese level in whole tops (Graham *et al.*, 1985 (cited by Reuter *et al.*, 1997).

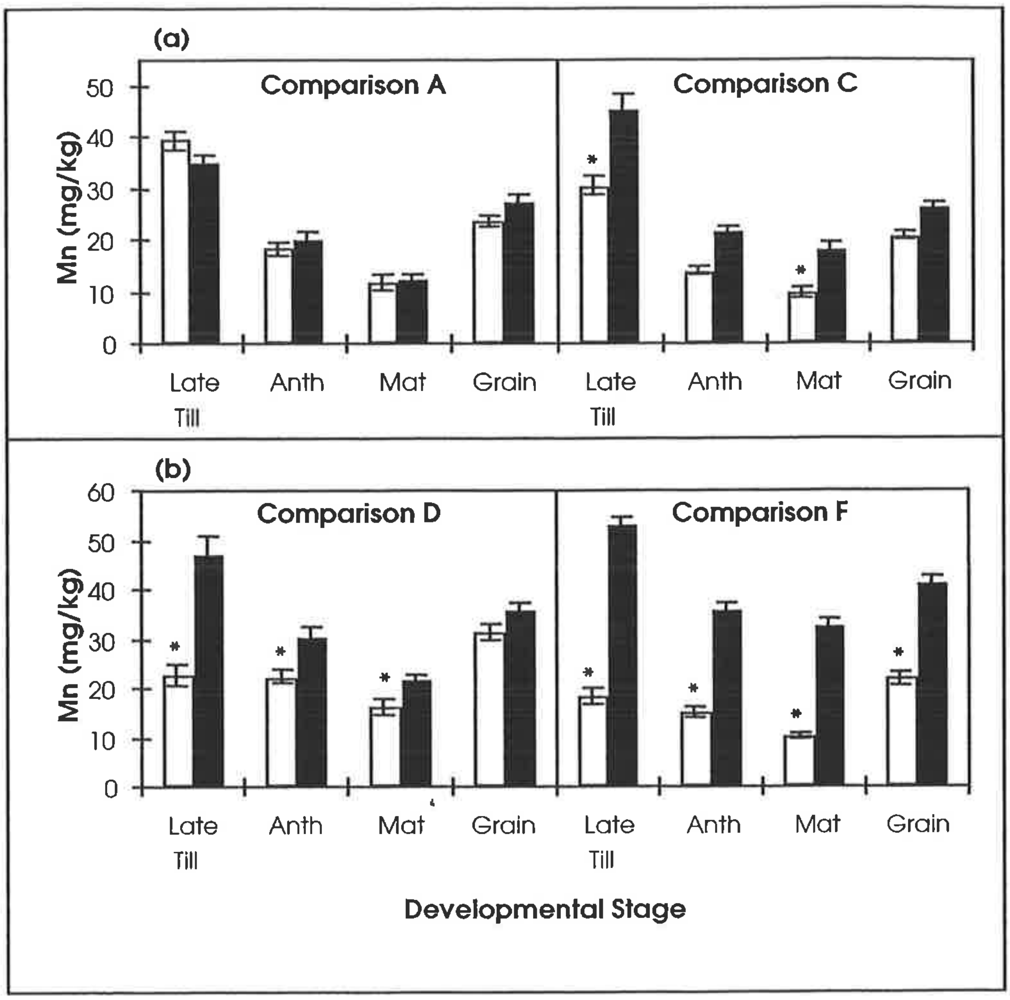


Figure 4.13 The manganese concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage (P<0.05).

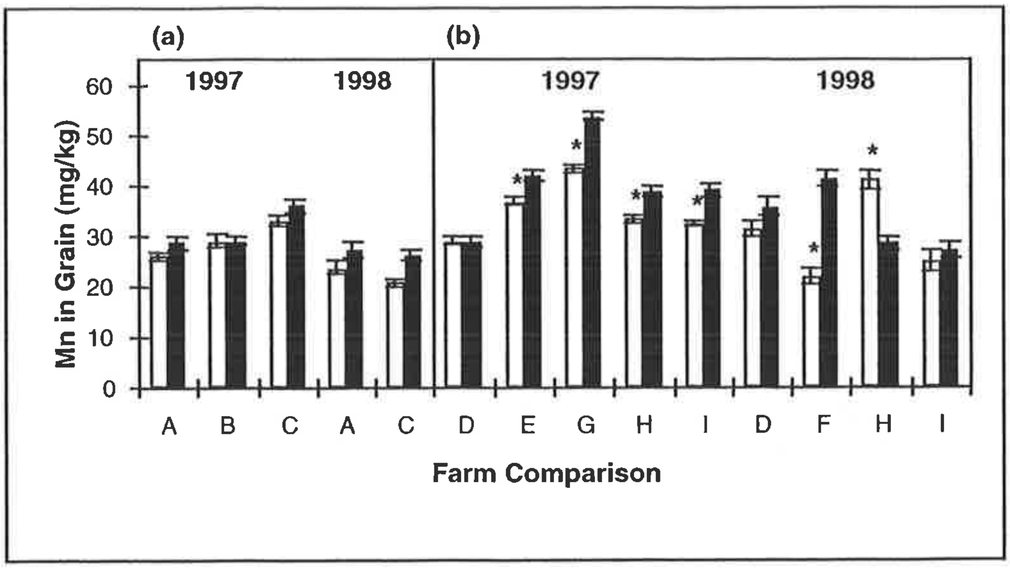


Figure 4.14 The manganese concentration in the grain of wheat grown organically (□) and conventionally (■) in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

4.3.6 Copper

No copper fertiliser was added by the conventional farmers. However, copper was present in the fertilisers applied to the organic systems (70-900 mg/kg, values quoted in sales catalogues or determined by external laboratory analyses in the absence of a sales catalogue) in all comparisons except for comparisons B and G in 1997. The organic systems, therefore, received 0.8-24 g of copper per hectare and in comparison A (1997), 159 g of copper per hectare was applied to the organic system.

In the high rainfall environment, copper concentrations in the soil were significantly greater in the organic systems compared with the conventional systems (Table 3.3 and Appendix 2).

Differences between the organic and conventional systems in terms of copper in the plant tissue at late tillering were somewhat inconsistent when the comparisons were viewed independently (Figure 4.15), although the organically grown plants had significantly greater copper concentrations by the mega-analysis (Table 4.1). The comparisons for which measurements were taken through the season, showed that the copper levels in the tissue of the organically grown plants were greater than or equal to those of the plants from the conventional system (Figure 4.16). The copper concentration in the grain was greater in the organically grown wheat or, in most cases, not significantly different to the conventionally grown wheat (Figure 4.17). However, the mega-analysis showed that the relatively small increase in copper of the organically grown wheat as compared to the conventionally grown wheat was highly significant (Table 4.1).

The level of copper in the plant tissue at late tillering was below the critical level in the organic system in comparison E in 1997 and comparisons D and F in 1998 and also in the conventional systems of comparisons D, F and I in 1998 (Figure 4.15). All of these farming systems diagnosed as copper deficient were from the low rainfall environment. However, the organic system from comparison B (1997) in the high rainfall environment was only marginally above the critical level of copper.

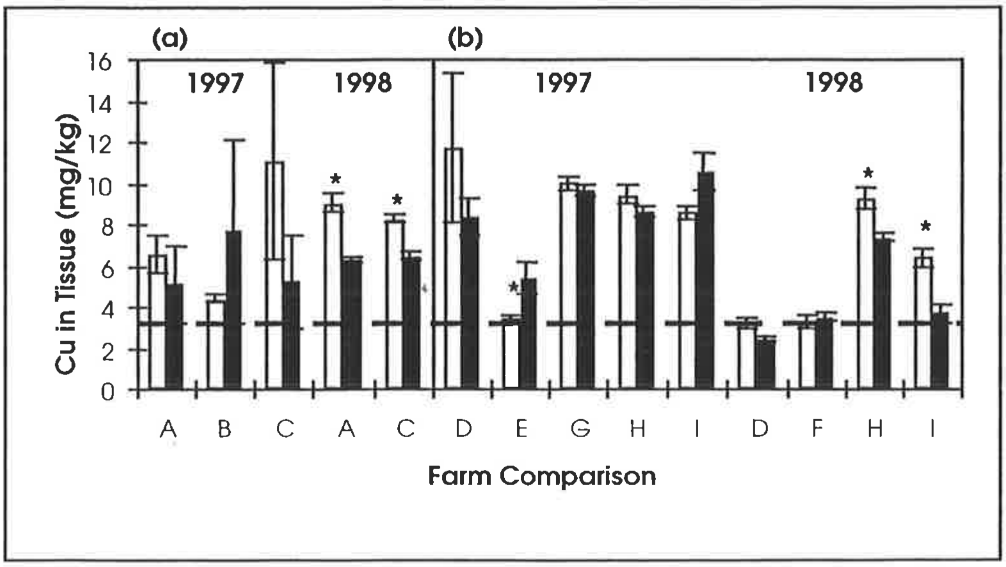


Figure 4.15 The copper concentration at late tillering of the whole tops of wheat plants grown in organic (☐) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$). The broken line is the critical copper level in whole tops (estimated based on Karamanos *et al.*, 1986 (cited by Reuter *et al.*, 1997)).

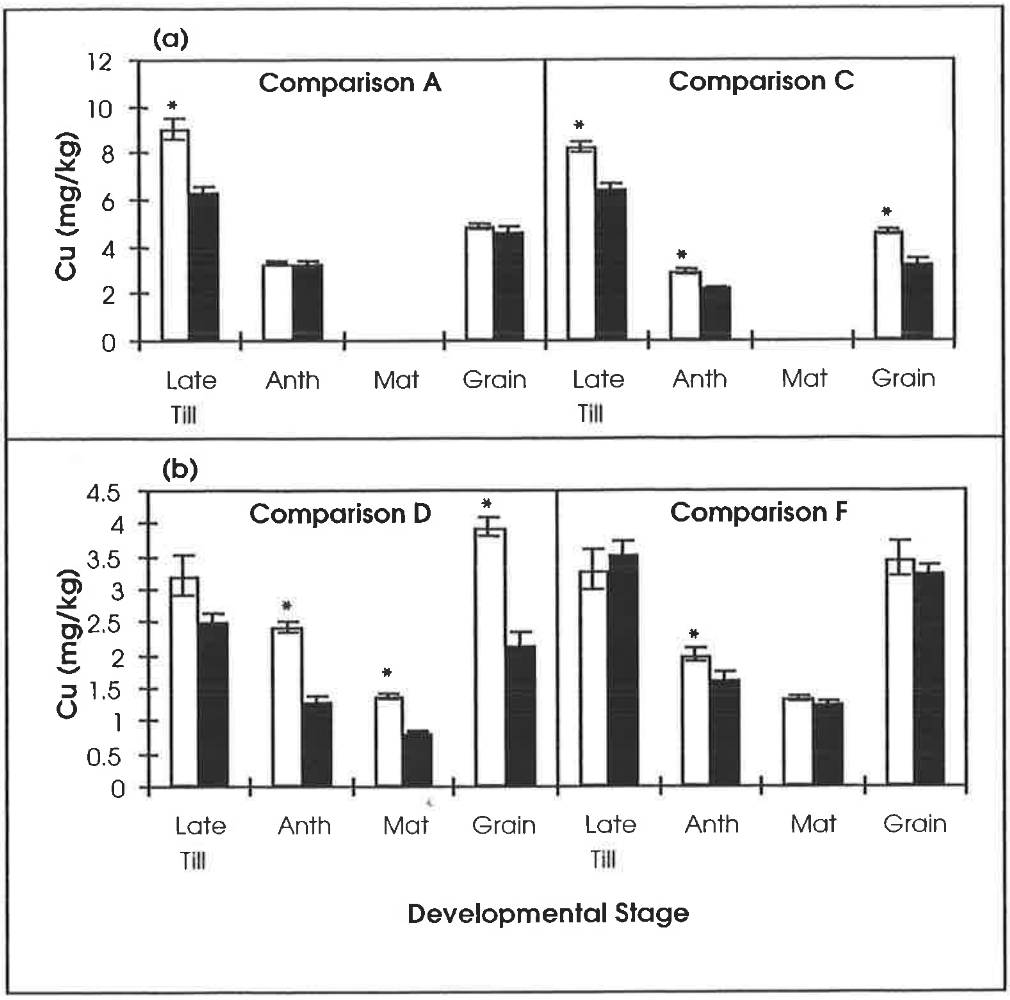


Figure 4.16 The copper concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of wheat plants grown in organic (□) and conventional (■) farming systems in high (**a**) and low (**b**) rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$).

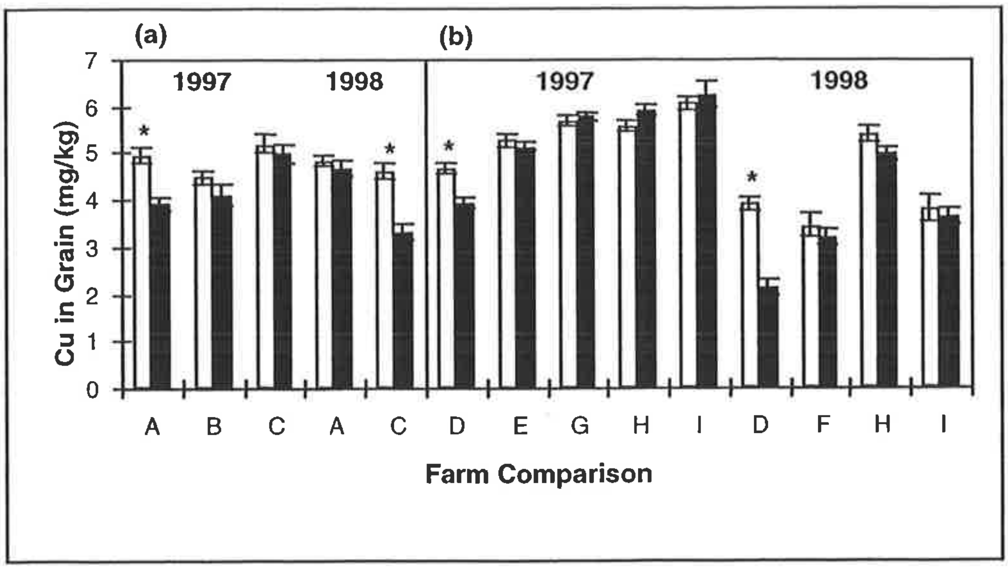


Figure 4.17 The copper concentration in the grain of wheat grown organically (☐) and conventionally (■) in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

4.3.7 Zinc

Zinc fertiliser was applied at rates between 1.2-2.5 kg per hectare at the time of, or prior to, sowing in the conventional systems of comparisons B, G and H in 1997 and comparisons H and I in 1998. It was applied at much lower rates (2-65 g/ha) in the organic systems of comparisons C, D, E, H and I in 1997 and comparisons A, C, D, F, H and I in 1998, as a component of the organic amendments applied. However, foliar zinc was applied to both the organic and conventional systems of comparison C in 1997 and to the conventional systems of comparisons I (1997) and A (1998) (Tables 3.1, 3.2 and Appendix 1). In fact, the foliar zinc applied to the latter system was applied within 48 hours prior to tissue sampling at late tillering, resulting in elevated zinc concentrations due to zinc sulphate remaining on the exterior of the leaves. For this reason, the results of the conventional system from comparison A in 1998 have been excluded from the ANOVA at the time of late tillering.

Zinc concentrations in the organically grown plant tissue at late tillering were mostly greater than or equal to those from the conventional system (Figure 4.18). The one exception was comparison D (1998) for which the conventionally grown plants had a higher zinc concentration, but even for this comparison, the organically grown plants had greater zinc concentration by the time of anthesis (Figure 4.19 (b)). In the grain, the zinc concentration in the organically grown wheat was greater than or equal to that of the conventionally grown wheat in all comparisons except for comparison I in 1997 (Figure 4.20).

There is still some debate regarding the critical level of zinc required by plants. Based on the critical zinc level used here, none of the systems are zinc deficient. However, there is some thought now that whole shoots with as much as 18-20 mg/kg zinc may be zinc deficient (R. Graham, *pers. comm.*) and, if so, zinc deficiency was indicated in plants from almost half of the systems, both organic and conventional (Figure 4.18), although more commonly in the conventional systems and in the low rainfall comparisons.

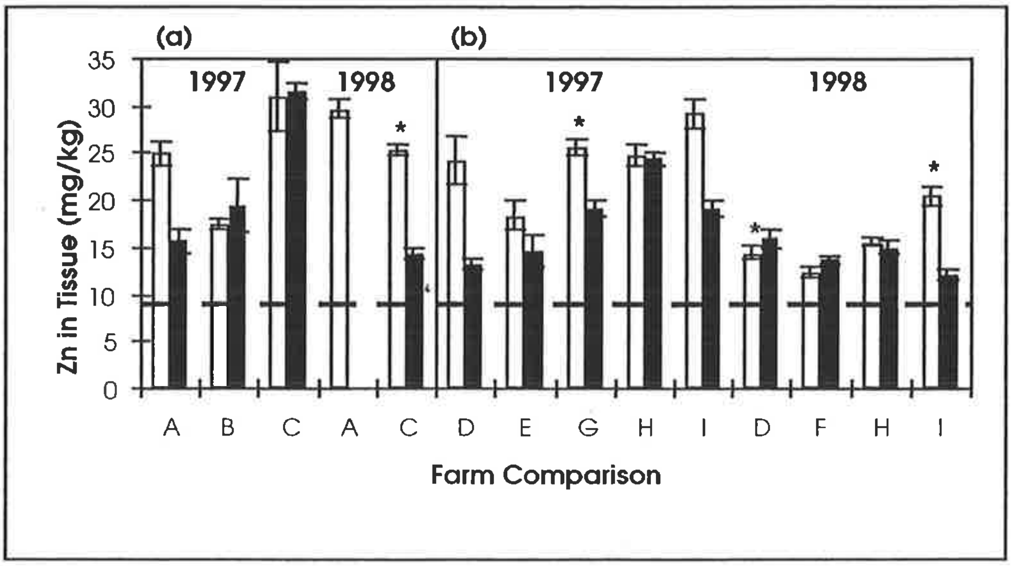


Figure 4.18 The zinc concentration at late tillering of the whole tops of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$). The broken line is the critical zinc level in whole tops (estimated based on Graham *et al.*, 1992 (cited by Reuter *et al.*, 1997)). Data from the conventional system of comparison A (1998) (averaged 110.2 mg/kg) were excluded from the ANOVA due to contamination by foliar zinc application prior to sampling.

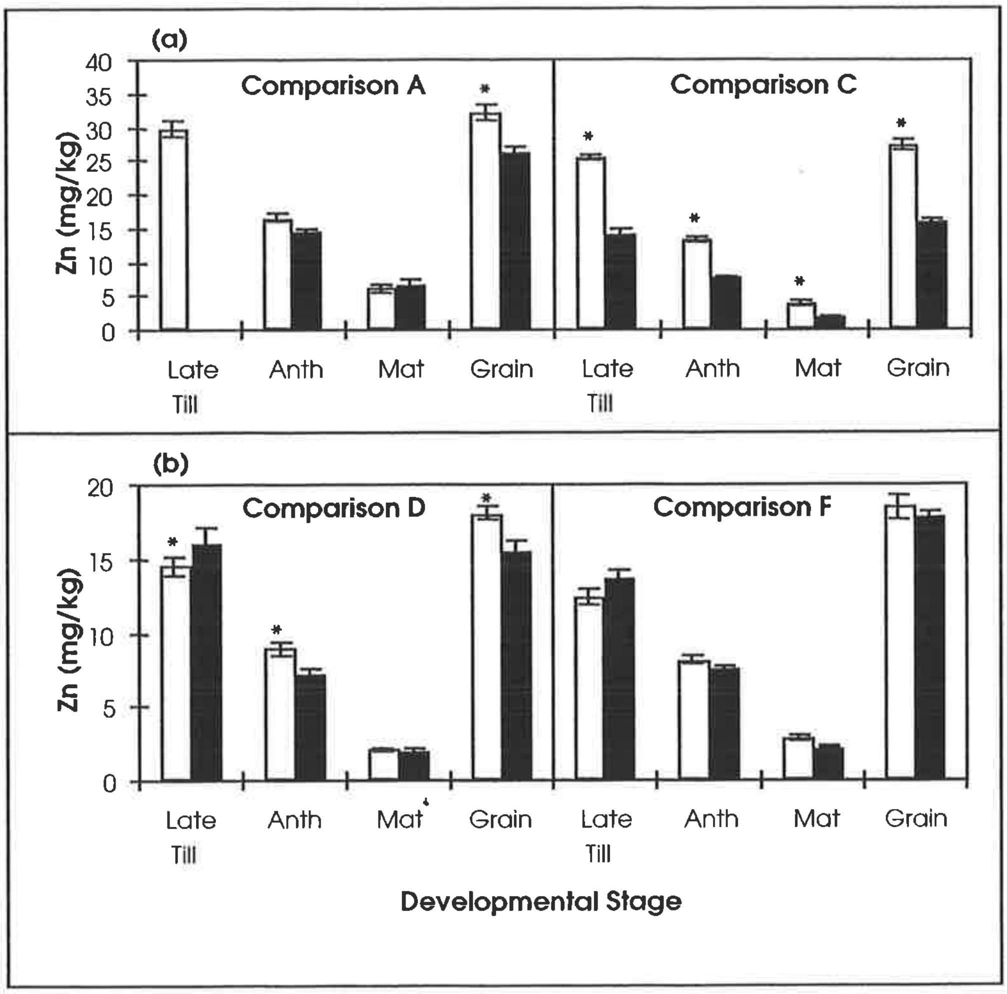


Figure 4.19 The zinc concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of wheat plants grown in organic (□) and conventional (■) farming systems in high **(a)** and low **(b)** rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$). Data from the conventional system of comparison A (1998) (averaged 110.2 mg/kg) at late tillering has been excluded from the ANOVA due to contamination by foliar zinc application prior to sampling.

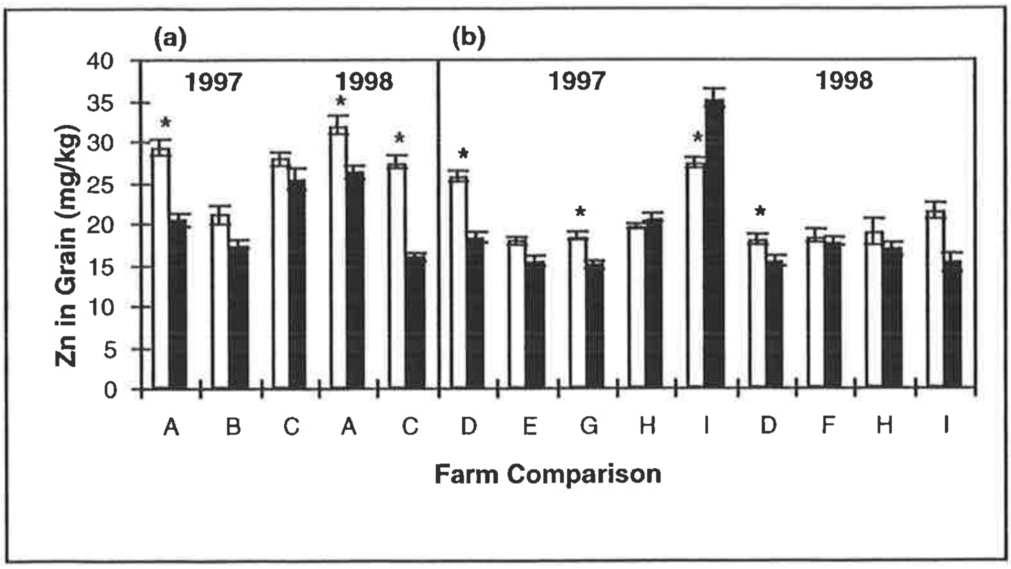


Figure 4.20 The zinc concentration in the grain of wheat grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

4.3.8 Protein Concentration and Amino Acid Composition of Grain

There were no significant differences in grain protein concentration between the organic and conventional systems in the individual comparisons (Figure 4.21). However, in the high rainfall environment, the grain protein concentration was overall significantly greater in the organically grown grain compared to the conventionally grown grain (Table 4.3). This was reflected in the organically grown grain also having greater concentrations of glutamic acid plus glutamine, glycine, histidine, isoleucine, leucine, phenylalanine, serine, tyrosine and valine (Table 4.3). However, in the low rainfall environments, the grain protein concentration tended to be greater overall ($P=0.073$) in the conventionally grown grain and the concentrations of almost all the essential amino acids were greater in the conventionally grown wheat compared to that grown organically.

In an attempt to account for the different total protein concentrations of the grains produced in contrasting systems and to identify qualitative differences in the protein caused by the farming system, the amino acids were expressed as a percentage of the total protein (Table 4.3; Figure 4.22). This showed that in the high rainfall environment, the protein of the conventionally grown grain had a greater proportion of alanine, aspartic acid plus asparagine and methionine. In the same environment, the protein of the organically grown grain had a greater proportion of glutamic acid plus glutamine and phenylalanine (Figure 4.22). However, in the low rainfall environments the only difference between the systems was that the grain from the organic system had greater methionine than the conventionally grown grain when it was expressed as a percentage of the total protein (Figure 4.22). There were no differences between the organic and conventionally grown wheat in terms of amino acid composition that were consistent over the two contrasting rainfall environments.

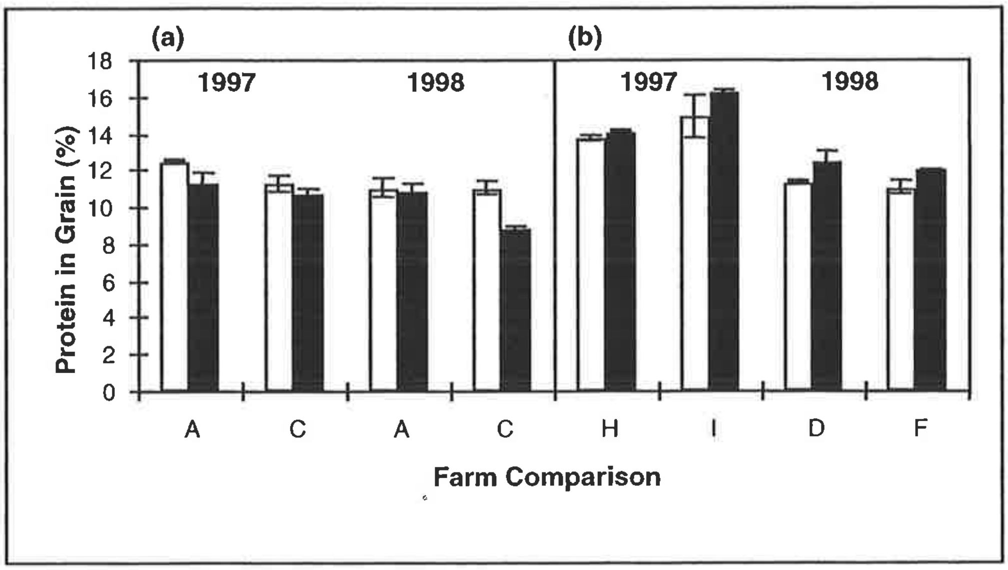


Figure 4.21 Protein concentrations (g/100 g DW) in wheat grain from organic (□) and conventional (■) farming systems in either high **(a)** or low **(b)** rainfall environments. There was no significant difference ($P>0.05$) between the organic and conventional systems in any of the comparisons.

Table 4.3 A summary of the ANOVA results of comparisons of amino acid concentrations of organically (Org) and conventionally (Conv) grown grain from high (HR) and low (LR) rainfall environments. Concentrations expressed either as g per 100g DW (protein amino acids) or as a percentage of the total protein. Quantitative data are included in Appendix 7.

Amino Acid	**g/100g DW**		**g/100g protein**	
	HR	LR	HR	LR
Ala	NS	Conv>Org	Conv>Org	NS
Arg	NS	Conv>Org	NS	NS
Asx	NS	Conv>Org	Conv>Org	NS
Cys	NS	Conv>Org	NS	NS
Glx	Org>Conv	Conv>Org	Org>Conv	NS
Gly	Org>Conv	Conv>Org	NS	NS
His	Org>Conv	Conv>Org	NS	NS
Ile	Org>Conv	Conv>Org	NS	NS
Leu	Org>Conv	Conv>Org	NS	NS
Lys	NS	Conv>Org	NS	NS
Met	NS	NS	Conv>Org	Org>Conv
Phe	Org>Conv	Conv>Org	Org>Conv	NS
Pro	NS	NS	NS	NS
Ser	Org>Conv	Conv>Org	NS	NS
Thr	NS	Conv>Org	NS	NS
Tyr	Org>Conv	Conv>Org	NS	NS
Val	Org>Conv	Conv>Org	NS	NS
Total Protein	Org>Conv	Conv>Org		

Asx signifies the combined results of aspartic acid and asparagine. Glx signifies the combined results of glutamic acid and glutamine. NS indicates no significant difference between farming systems (P>0.05).

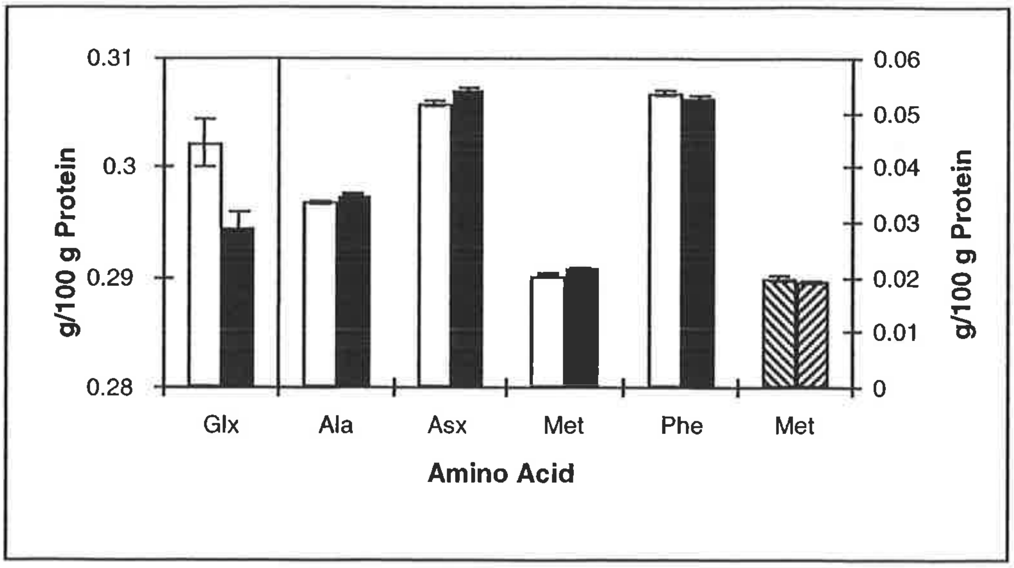


Figure 4.22 Concentrations (g/100 g protein) of amino acids which were significantly different ($P<0.05$) between wheat grown in high rainfall organic (□) and conventional (■) systems and between wheat grown in low rainfall organic (▧) and conventional ▨) systems. The vertical line indicates the reference axes for the amino acids.

4.3.9 VAM Colonisation

The VAM colonisation was significantly greater on the plants from the organic systems than those from the conventional systems. This was true in both high and low rainfall environments and for both varieties investigated (Figure 4.23). However, there was also a significant interaction between system and variety in terms of VAM colonisation. There was no difference between the varieties in the conventional system but in the organic system, Janz had greater colonisation than Dirk-48 (Figure 4.23 (b)).

There was no significant correlation, however, between the degree of VAM colonisation and the grain yield or the concentrations of phosphorus, copper or zinc in the tissue or grain of plants from either the organic or conventional farming systems (Table 4.4).

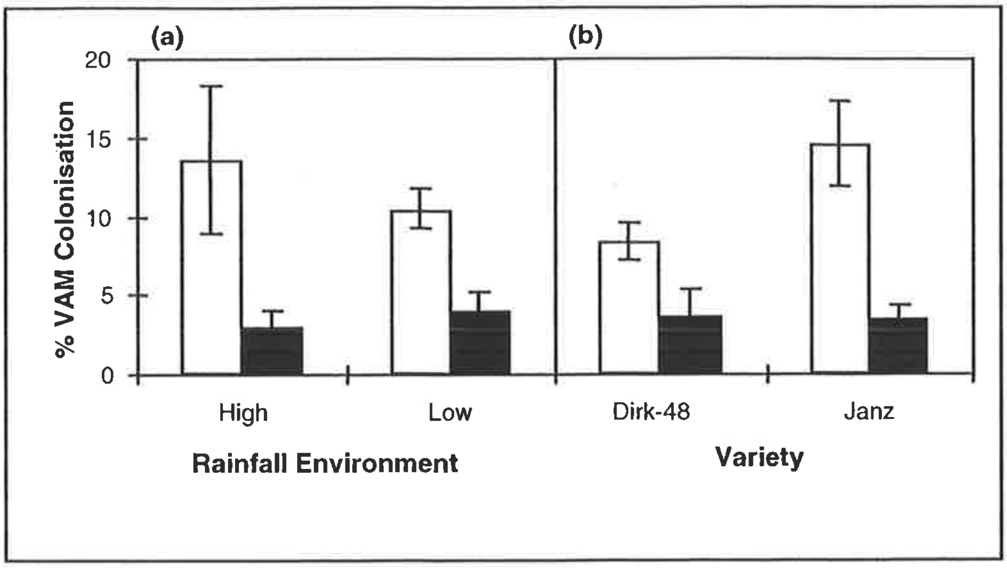


Figure 4.23 The percentage colonisation by VAM of plant root systems from organic (□) and conventional (■) farming systems (1998 comparisons only) in high and low rainfall environments (a) and comparing two wheat varieties (b).

4.4 Discussion

4.4.1 Phosphorus

The higher phosphorus concentration in plant tissue and grain from the conventional system (Figures 4.1-4.3) is not unexpected and was also observed by Derrick (1996). It is most likely due to a combination of two factors: Firstly, the lower phosphorus status of the organically, compared to the conventionally, managed soils (Tables 3.3 and 3.4), and secondly, the low solubility, and hence low availability, of the phosphatic fertilisers used in the organic systems (Weatherly *et al.*, 1988) compared with the readily soluble phosphate used in the conventional systems. Furthermore, none of the organic systems from the low rainfall environments had phosphatic fertilisers applied due to the inability of their alkaline calcareous soils to solubilize the phosphate rock. Low soil moisture in the low rainfall environments may also contribute to the insolubility of the phosphate rock (Weatherly *et al.*, 1988).

Table 4.4 Simple linear correlations (r) between percentage of the root system colonised by VAM and the grain yield and selected nutrient concentrations in tissue, at late tillering (LT), anthesis (A) and maturity (M), and grain (G) from the organic and conventional farming systems.

	n	**Organic** **(r)**	**Conventional** **(r)**
Grain Yield	12	+0.297	+0.090
Cu (LT)	12	-0.059	+0.515
Cu (A)	8	-0.100	+0.088
Cu (G)	12	-0.448	+0.349
Zn (LT)	11	+0.133	+0.095
Zn (A)	8	-0.003	+0.193
Zn (M)	8	-0.068	-0.006
Zn (G)	12	-0.008	-0.415
P (LT)	12	+0.266	-0.256
P (A)	8	+0.577	+0.409
P (M)	8	+0.510	+0.057
P (G)	12	+0.221	+0.384

None of the correlations is significant (P>0.05)

It has been found in Australia, and throughout the world, that extractable phosphorus levels in the soil are commonly lower in organic farming systems than in comparable conventional systems (Lockeretz *et al.*, 1980; Lengnick and King, 1986; Reganold *et al.*, 1993 (mixed farms); Penfold *et al.*, 1995; Deria *et al.*, 1996; Entz *et al.*, 1998; Waldon *et al.*, 1998; Derrick and Dumaresq, 1999). The results presented here show that the organic systems investigated in this study follow the same pattern. The only examples in the literature of organic systems that have greater extractable soil phosphorus than conventional systems are those for which animal manure is a major nutrient source (Fraser *et al.*, 1988; Reganold *et al.*, 1993). Such organic systems have been commonly used for intensive rather than extensive production, for example vegetable rather than wheat production.It appears that as well as extractable phosphorus, total soil phosphorus may be reduced, under some organic systems compared to conventional systems (Waldon *et al.*, 1998; Derrick and Dumaresq, 1999). However, some researchers have reported little difference in total soil phosphorus between the systems (Lockeretz *et al.*, 1980; Oberson *et al.*, 1993; Dann *et al.*, 1996) and still others, that the organically managed soil had significantly greater total phosphorus (Lengnick and King, 1986) due mostly to a higher level of organic phosphorus in the organically managed soil. This indicates that management techniques may be the key to differences between farming systems. Management techniques can differ quite a lot between organic systems and, therefore, in some environments and under certain management, the total soil phosphorus may be greater in comparison to the conventional system while in another environment and using different management techniques, it may be less than that of the conventional system.

Based on data for the South Australian wheat belt soils, the critical requirement for extractable phosphorus in the top soil (using the sodium bicarbonate extraction technique developed by Colwell (1963)) is about 21 mg/kg (Reuter *et al.*, 1995). Below this level, a significant growth and yield increase is expected in response to the addition of soluble phosphorus fertiliser. The levels of extractable phosphorus (Colwell) in the organically managed soils from the high rainfall environment varied between 9 and 20 mg/kg, compared to between 26 and 34 mg/kg for most of the conventionally managed soils (Table 3.3). Based on this, it can be predicted that plants from the organic systems in most comparisons, and the conventional system from comparison C in 1997 (14 mg/kg), were limited in their growth due to a lack of phosphorus. Likewise, in the low rainfall environment, all of the organic systems (except in comparison H (1997) which was also approaching deficiency) and the conventional systems from comparisons D and E (1997) and I (1998) had levels of soil phosphorus that pointed to phosphorus deficiency (Table 3.4). The conventional systems from comparisons H and I (1997) and D (1998) were also approaching phosphorus deficiency, assessed through soil phosphorus concentrations.

The tissue analyses at late tillering, however, do not reveal phosphorus deficiency in any of the conventional systems. Soluble phosphatic fertilisers were applied to every conventional system. In the conventional systems where phosphorus deficiency was indicated by the soil analyses which were taken mid season, the phosphate from the fertilisers may have already been taken up by the plants. The plants would then not show phosphorus deficiency because of the adequate phosphorus in the tissue. However, they may have exhausted the phosphorus supply later in the season during anthesis or grain development. This is not as important, though, as phosphorus supply early in the season when the phosphorus uptake by wheat plants is greatest (Romer and Schilling, 1986).

In the organic systems, the low rainfall comparisons with low soil phosphorus levels also had tissue phosphorus concentrations indicative of phosphorus deficiency in most cases. However, in the high rainfall environment, the phosphorus concentrations in the plants at late tillering were not below the critical level in the organic or conventional systems. Rather than an effect of rainfall, this result is probably due to the maintenance of the total soil phosphorus pool in the high rainfall organic systems through the addition of insoluble rock phosphate fertilisers, whereas the total soil phosphorus was not maintained where phosphatic amendments were not applied in the low rainfall organic systems. Therefore, although the extractable soil phosphorus pool in the high rainfall organic systems is not directly replenished through soluble fertiliser application, as in the conventional systems, it is replenished as the insoluble phosphate moves into the extractable pool. Because this movement from the insoluble to the extractable phosphorus pools is often mediated by plants or soil organisms through the production of enzymes or acidic root exudates (Hoffland, 1992), it is also quickly absorbed from the soil by plants or soil organisms, leaving the extractable phosphorus low in the soil of the organic system. However, plants can still obtain adequate phosphorus under these conditions. A low extractable soil phosphorus level is only of concern, therefore, if the rate of movement from the insoluble to the extractable phosphorus pool is not adequate to supply the growing plants with phosphorus. Based on the tissue phosphorus concentrations in the high rainfall organic systems, the supply of phosphorus is adequate, indicating that the rate of solubilisation of rock phosphate is sufficient. However, the low tissue phosphorus concentrations in many of the low rainfall organic systems reveals that, without replenishing the total phosphorus pool through the addition of rock phosphate, the solubilisation of unavailable soil phosphorus is not adequate.

The fact that the critical level for soil phosphorus, as reported in the literature (summarised by Moody and Bolland, 1999), indicated that the organically managed soils were phosphorus deficient, even when the plants did not manifest phosphorus deficiency, suggests that the criteria used to determine the critical level of phosphorus in soil may not be appropriate within organic farming systems. This suggestion was also made by Waldon *et al.* (1998) who measured low soil phosphorus but had healthy plants in their organically managed sites. It is possible that within organic farming systems, solubilisation of rock phosphate is more efficient than in conventional systems due to the influence of VAM (refer to sections 2.4.2 and 4.4.4) in organic systems. Another possible factor is the increased solubilisation of rock phosphate by microbes present in soils not treated by pesticides, compared with treated soils (Taiwo and Oso, 1997).

Lower levels of phosphorus in the grain, as for organically grown grain, may have agronomic implications for the farmer. Previous research has shown that high phosphorus concentrations in wheat grain are related to an increased biomass production of seedlings that is still evident in the field 67 days after sowing (Bolland and Baker, 1988; Derrick and Ryan, 1998). This growth advantage would be particularly important in organic systems where extractable soil phosphorus is low. Derrick and Ryan (1998) suggest that organic farmers should grade their grain by size, sowing the grain that is larger and therefore has a greater phosphorus content, compared to smaller grain with the same phosphorus concentration.

Lower phosphorus levels in grain can also have nutritional implications. Phosphorus deficiency in humans is rare, but phosphorus is essential in both plants and animals because of its structural role in DNA and important involvement in energy transfer.

4.4.2 Phytate, Magnesium and Potassium

The phytate concentration within the grain was greater in the conventionally compared with the organically grown grain (Figure 4.4). This is a reflection of the greater total phosphorus concentration in the conventionally grown grain, with which phytate concentration is highly correlated (Batten, 1986).

One of the roles of phytate is as a storage compound for phosphorus in the grain. Furthermore, phytate is thought to be involved with the storage of cations, for example magnesium and potassium, because phytate in grain is usually found as a magnesium or potassium salt (Batten and Lott, 1986). This may completely account for the higher concentrations of magnesium and potassium in the conventionally, compared to the organically, grown grain (Figures 4.8 and 4.11). It could be tested by growing low phytate mutant cereals under organic and conventional conditions and monitoring the magnesium and potassium concentrations in the grain compared to the grain phosphorus. Mutants of this type have been developed in corn, rice and barley using chemically induced single gene mutation techniques (Stelljes, 1996; Ertl *et al.*, 1998).

Phytate is also an anti-nutrient (refer to Chapter 2, section 2.4.4) and for that reason, the lower concentration of phytate in the organically grown grain is potentially beneficial for consumers. Cooking or processing can help to mobilise phosphorus from the phytate, but particularly for stock, where grain is consumed without processing, the non-phytate component of the phosphorus content is an indication of the bioavailable phosphorus. Raboy and Dickinson (1987) reported that when different rates of phosphorus were applied to soybeans, the increase in grain phosphorus was due almost entirely to phytate accumulation. This would mean that for the low phosphorus treatments, the proportion of non-phytate phosphorus would be greater than in the high phosphorus treatments. In the current study, however, the ratio of phytate phosphorus to non-phytate phosphorus in the wheat grain remains similar between the different farming systems (Figure 4.5). This reveals that there is no corresponding increase in available phosphorus with phytate reduction in the organically grown grain.

To find such an increase would be of interest to pig and poultry farmers (Stelljes, 1996; Ertl *et al.*, 1998) because, like humans, such animals have only low levels of the phytase enzyme to utilise the phosphorus in the phytate. Hence farmers must supplement pig and poultry feed with phosphorus even though there is a high phytate phosphorus content in grain based feed. The high phytate phosphorus in the feed results in a high phosphorus content of pig and poultry manure. This high phytate phosphorus is a useful phosphorus source for organic farms where composted manure is applied. However, it is also thought to contribute in many regions to the environmental problem of the eutrophication of waterways (Stelljes, 1996; Ertl *et al.*, 1998; Lott *et al.*, 2000).

Phosphorus availability (indicated by the ratio of phytate phosphorus and non-phytate phosphorus) is similar between organically and conventionally grown grain. However, lower grain phytate, as an anti-nutrient, could result in improved bioavailability of cations such as iron, zinc, magnesium and calcium from the organically grown grain. This is because phytate binds these cations in the animal gut and renders them less available. This possibility is further developed in Chapter 5.

Phytate has also been found to be beneficial in cancer prevention by reducing the indices of early risk markers for breast and colon cancer (Thompson and Zhang, 1991). The relationship of phytate to cancer may also be partly curative because phytate has been shown to reduce both the incidence and growth rate of tumours in rats (Jariwalla *et al.*, 1988), resulting in increased survival time. It is thought that the action of phytate in relation to cancer could be associated with its cation binding abilities because the cancer indices, tumour incidence and tumour growth were increased through the supplementation of rodents with magnesium, calcium and iron (Jariwalla *et al.*, 1988; Thompson and Zhang, 1991) but reversed through phytate supplementation. The implication, therefore, is that phytate could be either a beneficial or detrimental component of the diet, depending on whether the main aim is to avoid nutrient deficiency or cancer. This suggests that organically grown grain (lower in phytate than conventional) may not be a beneficial component of a cancer preventative diet from the perspective of phytate.

Both positive and negative nutritional effects of the grain also need to be considered in the context of the percentage of wheat products in the diet and the effects of processing the grain. These are discussed further in Chapter 6.

Neither magnesium nor potassium was found by Derrick (1996) to be significantly different between organically and conventionally grown grain. A comparison with much less scope, replication and control (O'Reilly, 1993) reported that potassium concentrations in organically grown wheat grain were significantly less than conventionally grown wheat (similar result to the current study) but that the organically grown grain had a greater magnesium concentration (opposite result to the current study).

In the comparison by Derrick (1996) of organic and conventional farming systems, magnesium concentrations in the plant tissue did not differ significantly between the farming systems at any measurement through the season. The average annual rainfall for the farms in the comparison by Derrick (1996) was comparable to the annual rainfall in the high rainfall comparisons of the current study. In the high rainfall comparisons of the current study there was also very little difference between the two farming systems in terms of magnesium concentration in the tissue through the season, but in the low rainfall comparisons the conventionally grown plants had greater magnesium concentrations than the organically grown plants (Figure 4.6). In contrast, it was in the low rainfall comparisons that the exchangeable magnesium in the soil was significantly greater in the organic systems (Appendix 2), and it was only in the organic systems that magnesium was applied to the soil. Therefore, the higher magnesium in the conventionally grown plants from the low rainfall comparisons was not due to higher soil magnesium in the conventional systems. Furthermore, because magnesium was applied to the organic systems (although at low rates) but not to the conventional systems, the higher magnesium concentrations in conventionally grown plants and grain suggests differences in magnesium uptake between the two farming systems. This may be due to a greater magnesium requirement by conventionally grown plants, or due to a lower availability of the magnesium present in the organic system.

Like magnesium, potassium concentrations in the plant tissue through the season seemed inconsistent between the two farming systems (Figure 4.9), even though it was significantly greater in the conventionally grown plants as determined by the mega-analysis (Table 4.1). In the comparison by Derrick (1996) of organic and conventional farming systems, potassium was significantly lower in the organically grown plant tissue at tillering. This is in agreement with the mega-analysis of the current study, but was not found to be so by Derrick (1996) at any other developmental stage.

The relatively small or inconsistent differences between organic and conventional farming systems in terms of magnesium and potassium concentrations in the tissue through the season, and the consistently higher concentrations of both of these nutrients in the conventionally grown grain, supports the view that the high levels of magnesium and potassium in the grain are linked to the high phytate levels in the same grain or *vice versa*.

4.4.3 Manganese

In a comparison of organic and conventional systems in New South Wales, Derrick (1996) found no significant difference in manganese concentrations in the plant tissue. However, he reported a non-significant trend towards higher manganese concentrations in the grain of the conventional system. In the current study, the same trend was shown in the grain and there was less difference in foliage manganese concentration between the farming systems in the high rainfall comparisons, which had similar average annual rainfall to the farms compared by Derrick (1996).

It has been reported that increasing phosphorus application results in reduced manganese concentrations in shoots, roots and seeds of soybean (Soliman and Farah, 1985) and rice (Basak *et al.*, 1982). In the current study, however, manganese concentrations were greater in the conventional system where soluble phosphorus was applied. Wang *et al.* (1995) found that shoot manganese in radish was generally higher when phosphorus was applied, either as soluble phosphorus or rock phosphate, in acid soil. However, when lime was also applied, the rock phosphate treatment resulted in only a small increase in shoot manganese compared with a much larger increase due to the application of soluble phosphorus. In the current study, the soils are alkaline and the results would therefore be most comparable to the effects reported by Wang *et al.* (1995) in the presence of lime. The much greater difference in the low rainfall comparisons between the organic systems (where no phosphatic fertiliser was applied) and the conventional systems (where soluble phosphorus was applied) may be due to the fact that the addition of rock phosphate to the organic systems in the high rainfall comparisons is slightly increasing the shoot manganese concentration so that the gap between the organic and conventional systems is less.

4.4.4 Zinc, Copper and VAM

Derrick (1996) reported significantly greater zinc and copper concentrations in organically, compared with conventionally, grown grain in New South Wales, as found in the current study. However, no difference was found in grain zinc (Pommer and Lepschy, 1985; Jorhem and Slanina, 2000) or copper (Pommer and Lepschy, 1985) concentrations of wheat grown under organic and conventional farming systems in Europe. This supports the observation that organic farming is different in Australia than it is in Europe where the concept evolved. Differences in climate, soil and farming practices (organic and conventional) may all contribute to differences in zinc and copper concentrations between organically and conventionally grown grain in Australia but not in Europe.

Although Derrick (1996) also found higher zinc and copper concentrations in organically, compared with conventionally, grown plants at tillering and anthesis, the differences were not statistically significant. In the current study, however, these same trends were statistically significant.

Unlike phosphorus, the lower zinc levels in the conventionally grown grain do not appear to be directly related to either low soil zinc or whether zinc fertiliser had been applied. In the high rainfall comparisons, the conventional systems contained greater (or equal) amounts of extractable soil zinc than the organic systems (Table 3.3), but the differences were not statistically significant. Zinc was added, as either granular or foliar fertiliser, to eight of the fourteen conventional systems but only one organic system. However, it was applied in low levels to most of the organic systems in a non-readily available form present in the rock fertilisers applied.

Conversely, extractable copper levels in the soil from the conventional systems in the high rainfall environment were less than or equal to those in the organic systems (Table 3.3). In the low rainfall environments there was no consistent difference in extractable soil copper between the farming systems (Table 3.4). No granular or foliar copper fertiliser was added to any systems, conventional or organic. However, like zinc, copper was present in low levels within the rock fertilisers added to most organic systems. The higher copper levels in the organically grown grain from the high rainfall comparisons may, therefore, be due, at least partly, to the copper status of the soils, maintained through the addition of copper in rock fertilisers. However, this does not adequately explain similar results in the low rainfall comparisons.

The greater biomass and grain production in most conventional compared with organic systems is likely to result in reduced concentrations of other nutrients due to the growth dilution effect (Jarrell and Beverly, 1981). Conversely, the greater zinc and copper concentrations in the organically grown grain and tissue could be due entirely to a concentration of these nutrients in biomass for which growth has been limited due to phosphorus deficiency or competition with weeds. In fact, there is no evidence among the tissue data at late tillering that indicates otherwise. In the grain there is also clear evidence of the presence of the concentration/dilution effect in most comparisons. However, there are also examples where the organic system gave greater yields than the conventional system, and yet the grain concentrations of zinc and copper were still greater in the organic system (comparisons A (1997) and D (1998)). This result suggests that there is a factor over and above the concentration/dilution effect that can cause greater zinc and copper concentrations in the grain of at least some of the organic systems, compared to the conventional system. Even in the comparisons where the concentration/dilution effect is clearly present, an alternative factor may be simultaneously present, causing either increased or decreased nutrient uptake in plants, but with the effect masked by the effect of concentration/dilution.

The factor responsible for greater zinc and copper concentrations in organically than conventionally grown grain could be present either in the organic system, enhancing zinc and copper uptake, or in the conventional system, blocking zinc and copper uptake.

In the organic system, a possible factor enhancing zinc and copper uptake is VAM symbioses. Certainly, VAM colonisation is greater in plants from the organic systems than those from the conventional systems (Figure 4.23). Other researchers have also found this to be so (Ryan *et al.*, 1994; Douds *et al.*, 1995; Dann *et al.*, 1996; Ryan, 1998; Eason *et al.*, 1999; Ryan and Ash, 1999; Mader *et al.*, 2000). Ryan *et al.* (1994) showed that this result is due largely to the application of soluble phosphorus within the conventional systems. However, researchers have also found that VAM species vary between the two systems (Douds *et al.*, 1995) and a greater growth response was obtained with spores from organic systems (Eason *et al.*, 1999). VAM associations are common in low phosphorus conditions and have been reported to enhance not only the uptake of phosphorus, but also zinc, copper, manganese and iron (Tinker, 1984; Kucey and Janzen, 1987; Thompson, 1990; Al-Karaki *et al.*, 1998) under appropriate conditions. In the current study, however, there was no significant correlation in either the organic or conventional system between the degree of VAM colonisation of plants and their concentrations of copper or zinc, or even phosphorus, in the tissue through the growing season or in the grain (Table 4.4). This result is contrary to reports in the literature and is probably due to the fact that the effect of VAM on nutrient uptake is confounded by other effects, particularly soil nutrient contents in combination with varietal nutrient efficiency and varietal VAM affinity. It could also be due to relatively low infection levels (less than 15% of the root length colonised) compared with 60-80% root length colonised, as reported by some researchers (Ryan *et al.*, 1994; Ryan, 1998; Eason *et al.*, 1999). However, Douds *et al.* (1995) reported 5-35% of root length (not wheat) colonised by VAM in soil from low-input farming systems, and Mader *et al.* (2000) reported less than 10% of the root length of wheat in the field to by colonised by VAM, even in low input systems. Further work would be required to elucidate if these levels of VAM are contributing, and the degree of contribution , within each system, towards tissue and grain nutrient concentrations.

In the conventional system there are two factors, herbicide application and the addition of soluble phosphorus fertiliser, which could be involved in reducing zinc and copper concentrations in the conventionally grown grain relative to that in the organic system.

Copper has not been reported as being affected by phosphorus fertiliser application, but as a divalent cation often found in competition with zinc, it may be similarly affected.

The application of herbicides, including metsulfuron-methyl (tradename Ally), diclofop-methyl (tradename Tristar) and chlorsulfuron (tradename Glean) which were applied to the conventional systems in comparisons A, C, D and H (1997 and 1998), have been shown to reduce plant nutrient uptake, including zinc and copper (McLay and Robson, 1992; Osborne *et al.*, 1993; Pederson *et al.*, 1994; Wheal *et al.*, 1998). However, the uptake of other nutrients such as manganese, potassium and phosphorus, have also been shown to be reduced by herbicide application (Osborne *et al.*, 1993; Pederson *et al.*, 1994) where the mode of the effect is by reducing root growth. The concentrations of these nutrients in the plants were greater in the conventional system than the organic and so it is unlikely that herbicide application is the cause of the reduced zinc and copper concentration in the conventionally grown grain unless zinc and copper are limiting factors. These nutrients, however, did appear to be limiting in many systems, organic and conventional (Figures 4.15 and 4.18), and for this reason, herbicide application within such conventional systems may have contributed to low zinc and copper levels in the plants. Although Osborne *et al.* (1993) report that the effect of herbicide on nutrient uptake is temporary and rarely seen in the grain concentrations, where nutrient supply (for example, zinc and copper) is inadequate the effect has been shown to be more permanent (Osborne and Robson, 1992). However, the grain nutrient concentrations have not been investigated in this context and more intensive work is required to determine the actual contribution that herbicide application has toward reducing grain zinc and copper concentrations in conventional systems that are low in zinc or copper.

The use of soluble phosphorus fertilisers has been shown to result in a reduction in zinc concentrations in both grain and plant tissue (Sharma *et al.*, 1968; Ragab, 1980; Singh *et al.*, 1986; Choudhary *et al.*, 1995), possibly through phosphorus interfering with zinc translocation to the shoots (Sharma *et al.*, 1968; Ragab, 1980; Van Steveninck *et al.*, 1993). This may occur due to the presence of phytate in the pericycle of wheat roots which has been shown to bind zinc (Van Steveninck *et al.*, 1993). The greater phosphorus uptake by plants in the conventional farming systems would be likely to result in greater phytate concentration in the root and hence greater binding capacity. Soluble phosphorus was applied to the conventional system of every comparison in the current study. Therefore, this mechanism is likely to be one of the factors, together with herbicide enhanced zinc deficiency in zinc deficient soils and VAM symbioses, contributing to the higher concentrations of zinc in the organically grown plants and grain.

4.4.5 Protein and Amino Acids

The protein concentration of organically grown wheat was greater overall than the conventionally grown wheat in the high rainfall comparisons of the current study but was less overall in the organic systems, compared to the conventional systems, of the low rainfall comparisons (Table 4.3). It has been shown to be lower (Haglund *et al.*, 1998), to have no real difference (Lockeretz *et al.*, 1981), or to be inconsistently different (Belderok, 1978) by other researchers. Raboy *et al.* (1991) showed that protein was positively correlated to phosphorus and phytate concentrations in the grain of winter wheat. In the current study, however, this relationship was not found in the high rainfall comparisons.

The amino acid composition of protein has been commonly investigated in conventional systems with treatments of soluble nitrogen fertiliser but rarely for other fertilisers. This is of interest because nitrogen fertiliser was applied at sowing to all of the high rainfall conventional systems and four of the nine low rainfall conventional systems, but to only one organic system. A number of studies have shown that with increasing application of nitrogen fertiliser, and hence increasing protein concentrations, proportions of glycine and threonine drop and increases are observed in proportions of proline, glutamic acid and phenylalanine (from Larsen and Dissing Nielsen, 1966; Dubetz and Gardiner, 1979; Dubetz *et al.*, 1979).

In the high rainfall comparisons of the current study, where nitrogen was added to the conventional systems, the protein concentration was lower in the conventional systems than when no nitrogen fertiliser was applied (the organic systems). This is due to the growth dilution effect because the protein yield per hectare was not significantly different between the systems. Even though no nitrogen was applied to the organic systems, the proportions of phenylalanine and glutamic acid (and glutamine) in the protein increased but there was no difference between the systems in terms of the proportions of proline, threonine and glycine in the protein (Table 4.3).

The lower proportions of alanine, aspartic acid (and asparagine) and methionine in the protein of organically (relative to conventionally) grown grain from the high rainfall comparisons (Figure 4.22 and Table 4.3) are not related to the differences in protein. These effects, therefore, may be effects of the farming system, although only present in higher rainfall (and hence lower protein) areas of wheat growing.

In the low rainfall comparisons, methionine as a proportion of the total protein is significantly greater in the organically than the conventionally grown grain (Figure 4.22 and Table 4.3). This is the only difference between the farming systems in the low rainfall comparisons and is exactly the opposite of the result in the high rainfall comparisons. For this reason, although there are a number of significant differences between the organically and conventionally grown grain, it cannot be concluded that any of these deviations from the amino acid composition of conventionally grown wheat will be consistently present in organically grown wheat. Belderok (1978) also found little difference in amino acid composition between biodynamically (organic) and conventionally grown wheat flour. Furthermore, even for the amino acids that are significantly different between the organically and conventionally grown wheat in the current study, there is little difference between the absolute values. Therefore, the biological effect of consuming grain that is minutely lower in, for example, phenylalanine, would probably be negligible even for sufferers of phenylketonuria.

4.5 Summary

- The first aim of this part of the study was to assess the nutrient status of organic systems in both high and low rainfall environments within South Australia.

In all comparisons, both the organic and conventional systems were adequately supplied with potassium, magnesium and manganese as determined by the critical level of these nutrients in the plant tissue at late tillering. There were, however, some examples of copper and zinc deficient sites amongst both the organic and conventional systems, but particularly in the low rainfall environments. Phosphorus deficiency was present, indicated by tissue phosphorus levels, in many of the organic systems but only in the low rainfall comparisons where no phosphorus was applied to the soil. In the high rainfall organic systems, rock phosphate was applied and this seemed to adequately supply the plants with phosphorus, even though phosphorus deficiency was implied by the extractable soil phosphorus levels.

- The second aim was to compare the grain phosphorus levels of organic and conventional systems under a range of management and environmental conditions.

Grain phosphorus was significantly lower in the organic system in almost all comparisons, in both high and low rainfall environments. This was reflected in lower grain phytate levels in the organic system, although the proportion of grain phosphorus comprising of phytate was similar between the two farming systems.

- The third aim was to identify nutrients other than phosphorus which have consistently different levels in organically and conventionally grown grain.

Magnesium, potassium and manganese were found to be significantly lower in the organically grown grain as compared to the conventionally grown grain. Magnesium and potassium are the major cations commonly present in grain phytate and, therefore, the elevated levels of these nutrients in the conventionally grown grain is thought to be associated with phytate concentration.

Zinc and copper were found to be significantly greater in the organically grown grain, due partly to the concentration/dilution effect in many comparisons. VAM colonisation was shown to be greater in the organic system, but due to confounding effects, the contribution of the fungi to the zinc and copper status of the plants was not clear. It is hypothesised that the zinc and copper may have been simultaneously boosted in the organically grown grain due to VAM associations and reduced in the conventionally grown grain, regardless of the applications of zinc fertiliser, due to the previously reported effect of soluble phosphorus fertiliser on zinc translocation and the herbicide induction of zinc and copper deficiencies in systems low in zinc or copper.

- The fourth aim was to investigate the effect of organic and conventional farming systems on protein concentration and amino acid composition of grain.

No consistent effect of farming system on protein concentration or amino acid composition of the grain was observed.

Chapter 5

Nutritional Value of Organically and Conventionally Grown Grain: Feeding Trial

5.1 Introduction

The nutrient composition of organically and conventionally grown wheat was investigated in Chapter 4. It was found that the organically grown grain mostly had a lower phosphorus concentration, reflected in lower phytate, and lower magnesium, potassium and manganese concentrations than conventionally grown grain. Magnesium and potassium in the grain are usually associated with phytate (Batten and Lott, 1986). Both copper and zinc were found to be mostly in higher concentrations in the organically grown grain.

It has long been established that the bioavailability of zinc, to animals and humans, is reduced with the presence of phytate in the diet (House *et al.*, 1982; Flanagan, 1984; Turnland *et al.*, 1984). This raises the issue in the current study of comparative zinc bioavailability between the organically and conventionally grown grains. It is possible that the lower phytate and simultaneously higher zinc concentrations in the organically grown grain, compared to that conventionally grown, will result in greater bioavailability of zinc.

Zinc is extremely important in the nutrition of animals and humans (Berdanier, 1998). The involvement of zinc in DNA synthesis renders it critical for growth and its presence in many enzyme systems makes zinc important for metabolism. Zinc has also been linked to cognitive function, immune response (Fraker *et al.*, 2000), membrane integrity (O'Dell, 2000) and bone metabolism (Saltman and Strause, 1993; Holloway *et al.*, 1996). Furthermore, zinc deficiency is thought by some to be as common as iron deficiency, particularly in developing countries (Gibson and Ferguson, 1998). In addition to this, there is evidence that zinc and iron are antagonistic when one is supplemented without consuming food at the same time as the supplement is taken (Rossander-Hulten *et al.*, 1991; O'Brien *et al.*, 2000). However, when the supplement is taken with food or supplied through the food matrix, the antagonism is reportedly absent (Whittaker, 1998; Rossander-Hulten *et al.*, 1991).

Although compositional characteristics of grain can be readily determined using analytical techniques, the nutritional value or bioavailability of the grain components can only be determined accurately using a feeding trial. Therefore, in the current study a feeding trial was undertaken using rats to evaluate the bioavailability of zinc from the organically and conventionally grown wheat, and with the following specific aims:

- To determine if the combined effect of lower zinc and higher phytate in the conventionally, compared to organically, grown wheat will induce zinc deficiency in rapidly growing rats, and
- To investigate the effect of the lower phytate and higher zinc concentrations in the organically grown grain on iron and calcium metabolism in the rat, including bone composition.

5.2 Methods

5.2.1 Animals

Male weanling albino Wistar rats (*Rattus norvegicus* L.) were used for this experiment. They were aged 23 days (weighing 44.6-62.5 g) when treatments were applied. Throughout the experimental period, rats were housed individually in metabolic cages made from stainless steel and polypropylene plastic and kept in a controlled environment room with temperature ranging from 20-26 •C and light from 0700 in a 14:10 light:dark cycle. Cage position was re-randomised every 14 days.

All work reported here was approved by The University of Adelaide Animal Ethics Committee (Project No: W/06/99).

5.2.2 Diet Formulation

Diets were based on a modification of the AIN-93G purified diet for rodents (Reeves *et al.*, 1993). One diet (SYN) was formulated closely to the AIN-93G synthetic diet (details in Appendix 8), using casein as the protein source, so that results could be related to existing literature. Because of the focus on zinc, zinc was extracted from the casein by washing with EDTA three times, rinsing with deionised water each time to remove the EDTA (method used to extract zinc from soy flour (Record *et al.*, 1986; Lee *et al.*, 1993)). This succeeded in reducing the zinc concentration from around 80 mg/kg to 4 mg/kg.

Four diets (organic (ORG), organic with phytate-phosphorus added to the level in the conventional diet (ORG+PHYT), conventional (CONV) and conventional with zinc added to the level in the organic diet (CONV+Zn)) were formulated using wheat as the main protein and carbohydrate source and a contributor to mineral concentrations (details in Appendix 8). Mineral concentrations were balanced between the four wheat diets with the exceptions of zinc and phytate-phosphorus (Appendix 8).

The wheat used in the diets was of the variety Janz, sourced from the organic and conventional plots (comparison C) grown at Wolseley in the 1998 season (Chapter 3 and Appendix 1). This variety was selected because it was representative of the other varieties included in the field experiment with regard to zinc and phosphorus concentrations in the grain (Figure 5.1). Another reason for selecting Janz was that it is a variety that is actually grown by both organic and conventional farmers in South Australia, and is therefore representative of the grain eaten by consumers.

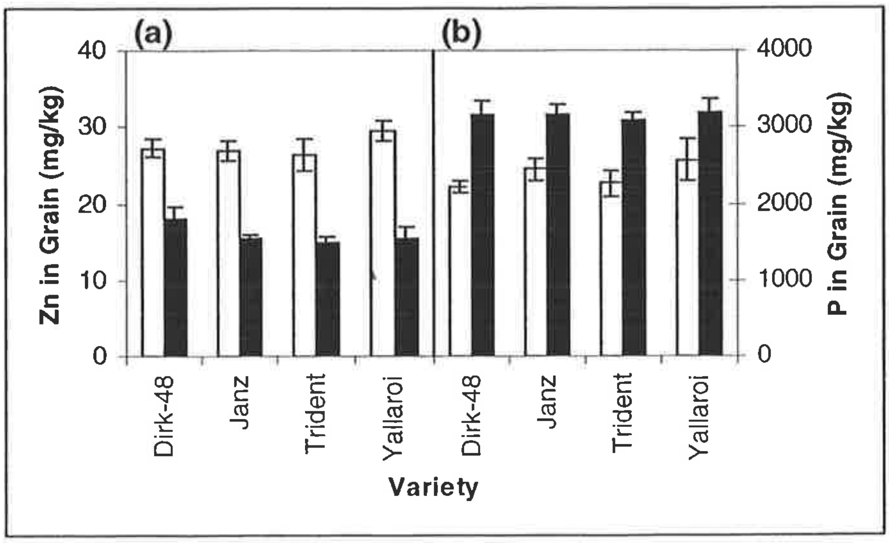


Figure 5.1 Grain concentrations of zinc (**a**) and phosphorus (**b**) in the selected varieties of wheat grown organically (□) and conventionally (■) in comparison C at Wolseley in 1998.

5.2.3 Nutrient Absorption

Five rats were randomly allocated to each of the five dietary treatments so that the mean initial weight of each group varied no more than 2.0 g.

Food and water (milli-Q) were supplied to rats *ad libitum* during the 42 day experimental period. Animals were weighed every 2 days and food and water consumption and urine and faeces excretion recorded. Samples of urine and faeces were collected every 2 days. Urine was stored at -80 •C and faeces were freeze dried, ground and then stored at room temperature. Both urine and faeces were digested using nitric acid and analysed for elemental composition using ICPAES (Appendix 9).

Apparent absorption of each nutrient from the feed was calculated as intake minus faecal excretion of that nutrient, expressed as a percentage of intake (ie. fractional apparent absorption). The nutrient retention from the diet (mg/day) was calculated by subtracting total excretion of each nutrient (faecal and urinary) from the nutrient intake.

Rats were randomly allocated to be killed on either day 41 or day 42 so that each dietary treatment was evenly represented on both days. All endpoint results will be henceforth referred to as day 42. It is to be noted that day of kill was not a statistically significant covariate when included in the ANOVA. Rats were euthanased by 1.0 ml lethal injection of Lethabarb (active ingredient Sodium pentobarbitone 325 mg/ml). At the time of death, the right hand side (dorsal view) hind limb was retained and frozen at -80 •C until the femur was dissected out at a later date. The length and mass of each femur were recorded and the nutrient composition analysed using ICPAES (Appendix 9). The liver and muscle *(Longissimus dorsi)* were also retained at time of death, snap frozen using liquid nitrogen and stored at -80 •C until required. Each were analysed for elemental composition using ICPAES (Appendix 9).

5.2.4 Blood Sampling

Blood samples were taken from the tail vein on days 16 and 30. Xylocaine gel was used as anaesthetic. On day 42, larger blood samples of approximately 5 ml were taken from the vena cava under anaesthesia. The blood was collected in tubes rinsed with Heparin (5000 U/ml) and was centrifuged to separate the red blood cells from the plasma, which was retained for elemental analysis using ICPAES (Appendix 9). At day 42, the percentage of red blood cells was determined using microhaematocrit tubes spun for five minutes on a Cellokrit.

5.2.5 Breath Sampling

The usefulness of breath analysis is outlined in Appendix 11. The purpose of analysing the breath of rats in this study is threefold: Firstly, to measure the rate of passage of substrate through the gastrointestinal tract; secondly, to determine the degree of substrate malabsorption due to the different diets; and thirdly, to compare quantitatively the gases present in the breath, as an indication of diversity within the bacterial population in the colon.

Breath samples were collected on days 30, 39 and 42 (Appendix 9 for details). On days 30 and 42 single breath samples were taken per rat and analysed for hydrogen and methane content using a Quintron Gas Chromatograph calibrated against known gases within defined hydrogen and methane concentrations.

On day 39, an additional series of breath samples were taken to determine rate of gastric emptying and fermentation rate. The rats fasted for at least 12 hours before the breath samples were taken. Immediately before breath sampling, the rat was gavage fed a lactulose meal (0.66 g/kg body weight) laced with ^{13}C labelled octanoic acid (equivalent of 10 μl/kg body weight). Four minute breath samples were taken at 0 minutes and every fifth minute for a period of 30 minutes and then every 30 minutes for a further 180 minutes. Additional samples were taken at 0 minutes and every subsequent 30 minutes to be analysed for hydrogen and methane content. The primary samples were analysed for carbon ratios using an isotope ratio mass spectrometer (Automated Breath ^{13}Carbon Analyser 400). Small intestinal transit time was calculated using the time at which half of the administered ^{13}C was exhaled ($T_{1/2}$) as the initial reference point. The $T_{1/2}$ was determined using the methods described by Ghoos *et al.* (1993). The second reference point was established as the time at which the hydrogen content of the breath (ppm/15 min/l of air in chamber) had increased 10 ppm above the baseline. This point (T_{caecum}) was representative of the entrance of the substrate into the caecum. The small intestinal transit time was the time taken for the substrate to pass between these two points.

5.2.6 Statistical Analysis

All statistical analyses were carried out using GENSTAT 5 unless otherwise indicated. For each data set, an ANOVA was carried out on the data and Tukey's Multiple Comparison Test (or the Tukey Kramer Test when data was unbalanced due to missing values) was used to determine significant difference between treatments at the five percent level.

Where normality of data was questionable or assumptions of even variance not met, data was transformed to achieve homoscedasticity (homogeneity of variance). In the case where transformations were used, the statistics from the transformed data are presented. However, the back transformed values are also included for ease of interpretation.

In some cases, data were also analysed by pooling the ORG and ORG+PHYT treatments and the CONV and CONV+Zn treatments into ORG(+) and CONV(+) treatments respectively. These were then compared with the SYN dietary treatment to determine the effect of the basal grain source in the diets. This was done in an attempt to determine effects caused by an inherent difference between the organic and conventionally grown grains, which had not been detected and hence not accounted for in the balancing of the diets.

For some of the multiple time point data sets (for example, faeces production and rat mass) the effect of the dietary treatment by the end of the 42 days was determined by analysing the final four data collections before the fast (ie. days 31, 33, 35 and 37). In this case, the four time points were treated as secondary replicates. Where this method of analysis was applied, it is indicated by stating that the data from days 31-37 were used.

The data sets for feed and water consumption, faeces and urine production, nutrient absorption measurements for zinc, calcium and iron, and breath hydrogen production over time on day 39 were analysed additionally in order to account for individual variation between the rats in each treatment group and to determine the effect of the diets over time. In this analysis, using ASREML, an extended linear mixed model was fitted to the data set (based on Verbyla *et al.*, 1999) and included terms, with fixed and random components, for time, rats and diets. Examples of fixed effects are the effect of the diets and the effect of the diets over time. Examples of random effects are the effect of the inherent variation between rats and the linear effect of each rat over time. Orthogonal contrasts were used on these data sets where there were significant fixed effects in order to determine which diets were significantly different from others.

5.3 Results

5.3.1 Feed Intake and Excretion

There was no significant difference in feed consumption between the five diets (Figure 5.2) except for feed consumption on the days following blood sampling (days 17 and 31). Feed consumption on these days, when expressed as a percentage of that consumed at the previous measurement, was significantly lower for the rats consuming the ORG+PHYT diet than rats consuming the other diets (Figure 5.3). There was no significant difference in the reduction of feed consumption between the other diets.

By the end of the experiment (using data from days 31-37), the rats fed the SYN diet excreted significantly less faeces (fresh weight and dry weight) than rats fed the grain-based diets (Table 5.1). The fresh weight, dry weight and water content of faeces excreted by rats fed the ORG(+) diets were significantly greater than for those rats fed the CONV(+) diets (Table 5.1).

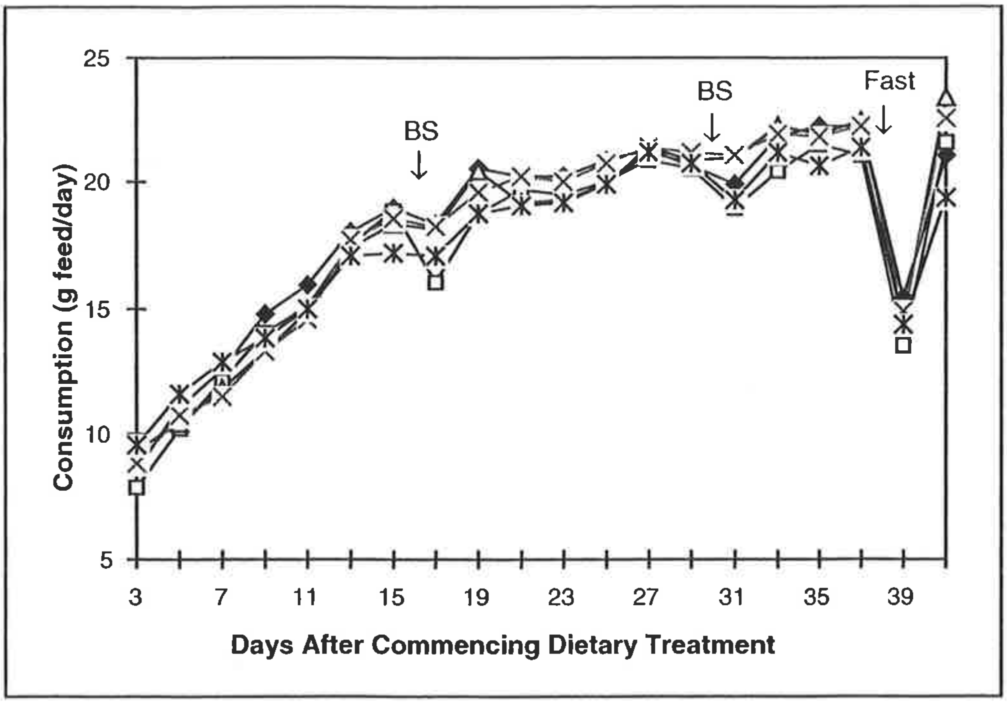


Figure 5.2 Feed consumption of rats fed organic (ORG, ♦), organic with phytate added to the level of the conventional (ORG+PHYT, □), conventional (CONV, Δ), conventional with zinc added to the level of the organic (CONV+Zn, ×) or an AIN-93G modified (SYN, ✶) diet. Blood sampling (BS) is indicated and also a 12 hour fast on day 38-39. When comparing treatments within one time point, the standard error of difference is 2.69.

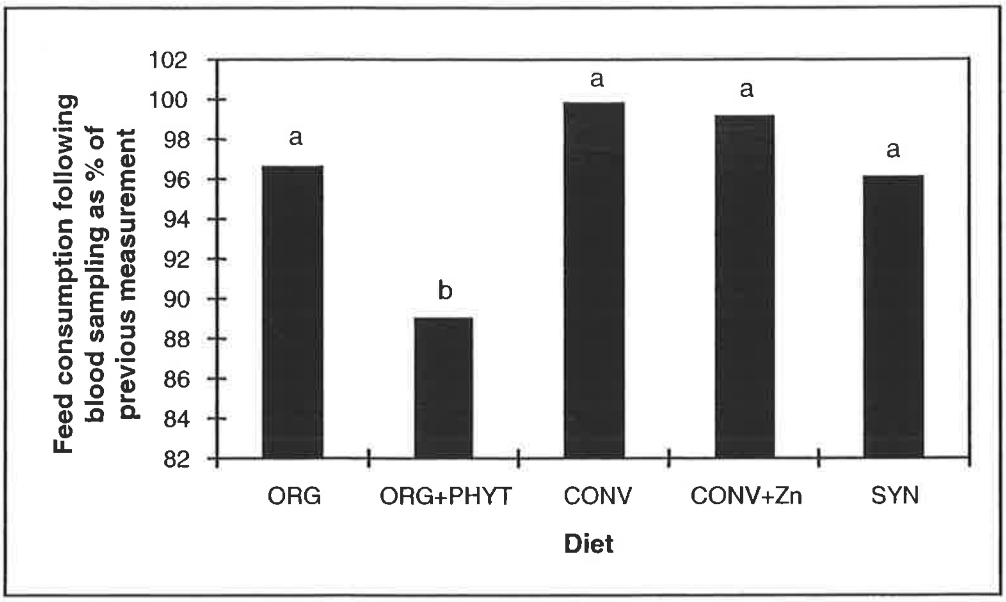


Figure 5.3 The effect of blood sampling on feed consumption of rats. The abbreviations of the treatment diets are defined in Figure 5.2. Treatments with the same lower case letter are not significantly different (P<0.05).

Table 5.1 Faecal excretion (g/day) by rats using data from days 31-37 of the dietary treatments* (n=20).

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
Faecal Excretion (FW)**	2.91^{a}	6.01^{c}	5.89^{c}	4.74^{b}	5.08^{b}	P<0.001
SYN, ORG(+), CONV(+)	2.91^{a}	5.95^{c}		4.91^{b}		P<0.001
Faecal Excretion (DW)**	1.40^{a}	2.19^{c}	2.21^{c}	1.99^{b}	2.09^{bc}	P<0.001
SYN, ORG(+), CONV(+)	1.40^{a}	2.20^{c}		2.04^{b}		P<0.001
Faecal Water Content (%)	0.51^{a}	0.63^{d}	0.61^{c}	0.58^{b}	0.58^{b}	P=0.002
SYN, ORG(+), CONV(+)	0.51^{a}	0.62^{c}		0.58^{b}		P<0.001

*SYN (synthetic, modified AIN-93G), ORG (Organically grown grain base), ORG+PHYT (Organically grown grain base plus phytate to the level of conventionally grown grain), CONV (Conventionally grown grain base) and CONV+Zn (Conventionally grown grain base plus zinc to the level of organically grown grain). **These values are adjusted for the significant covariates of water and feed consumption. ^{abcd}Means within the same row followed by the same lower case letter are not significantly different.

Based on the extended linear mixed model (section 5.2.6), there is a highly significant effect of feed consumption of individual rats on faeces excreted. With feed consumption included in the model of faeces production, there were also significant differences among diets in the changes in faeces production over time. Rats fed all diets except ORG have an almost parallel pattern of faeces production whereas rats fed the ORG diet have a greater rate of increase (greater slope) in faeces production over time (Figure 5.4). In the first few days of the treatment diets, ORG fed rats excreted a similar amount of faeces as rats fed the CONV diet but faecal production steadily increased to a level that was greater than the ORG+PHYT fed rats at the end of the experiment.

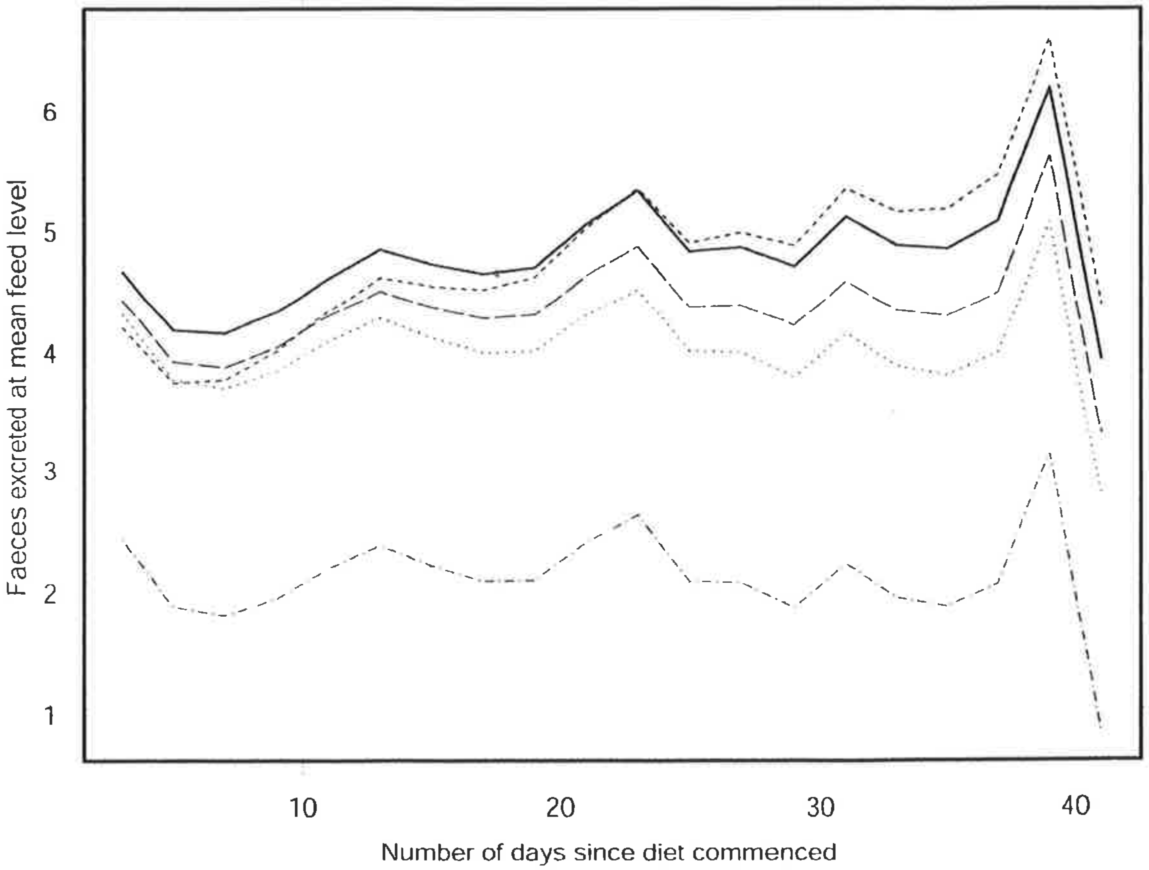


Figure 5.4 The final linear model of FW faeces production (g/day, adjusted for the covariate of feed consumption) by rats fed over a period of 42 days. The fitted graph is shown at the average feed consumption of 17.8 g/day. The dietary treatments (abbreviations defined in Figure 5.2) include ORG ( ), ORG+PHYT ( ), CONV (......), CONV+Zn ( ) and SYN ( ).

There were no significant differences between the diets in terms of the length or mass of the rat femurs (Table 5.2). However, there was a non-significant trend (P=0.093) towards the rats fed the SYN diet having heavier femurs than those fed the ORG+PHYT diet. Similarly, there was a non-significant trend (P=0.058) toward the rats fed the SYN diet having longer femurs than those fed the ORG+PHYT and ORG diets.

Both water consumption and urine production varied greatly, both between individual rats and for each individual over time. There was no consistent difference in water consumption, urine production or urine production/water consumed between the five diets (Appendix 10).

5.3.2 Growth

There were no significant differences between the five dietary treatments in terms of the growth rate of the rats, expressed as either actual growth rate (weight gain/day), relative growth rate (weight gain/day/weight at previous measurement) (Appendix 10) or as mass (adjusted for initial mass) at the end of the experiment (days 31-37) (Table 5.2).

The five dietary treatments resulted in rats exhibiting no consistent difference in the feed efficiency ratio (weight gain/feed consumed) (Appendix 10).

5.3.3 Zinc

The rats fed the SYN diet had a significantly greater zinc intake than those fed the grain-based diets. The rats fed the CONV diet had a significantly lower zinc intake than the other dietary treatments (Table 5.3). Similarly, the total zinc excretion (urinary plus faecal) of the rats fed the SYN and CONV diets was significantly greater and less, respectively, than the other dietary treatments (Table 5.3).

The mean urinary zinc excretion was significantly greater for those rats fed the SYN diet compared to all of the other diets (Table 5.3). Additionally, the rats fed the CONV diet had significantly less zinc in their urine than those fed the CONV+Zn diet. There were no significant differences in mean urinary zinc between the other dietary treatments. There were differences between the grain-based diets, however, in the change in urinary zinc over time with the rats fed the ORG and ORG+PHYT (ORG(+)) diets having a significantly steeper reduction in urinary zinc excretion towards the end of the experiment compared with the rats fed the CONV and CONV+Zn (CONV(+)) diets (Figure 5.5).

Table 5.2 Body mass (n=20, using data from days 31-37) and femur dimensions (n=5) of rats fed the treatment diets[*] for 6 weeks.

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
Rat Body Mass[**] (g)	295	291	269	288	284	NS
Femur Mass (g)	0.513	0.465	0.437	0.470	0.477	NS P=0.093
Femur Length (mm)	34.0	32.5	32.4	33.4	33.7	NS P=0.058

[*] Abbreviations of dietary treatments are defined in Table 5.1. [**] These means are adjusted for the significant covariate of initial mass. NS indicates that there is no significant difference between the diets $P<0.05$.

Table 5.3 Mean zinc balances over 6 weeks (n=100; 5 rats, 20 measurements) in rats fed the treatment diets*.

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
Zn Intake (mg/day)	0.626^{c}	0.439^{b}	0.446^{b}	0.288^{a}	0.464^{b}	P<0.001
Total Zn Excreted (mg/day)	0.392^{c}	0.234^{b}	0.229^{b}	0.122^{a}	0.266^{b}	P<0.001
Zn in Urine (mg/day)	0.023^{c}	0.013^{b}	0.014^{b}	0.009^{a}	0.016^{b}	P<0.001
Fractional Apparent Absorption of Zn ** (%)	41.87^{a}	51.28^{b}	52.79^{b}	61.54^{c}	47.30^{b}	P<0.001
Zn Retention* (mg/day)**	0.243^{c}	0.213^{b}	0.225^{b}	0.169^{a}	0.204^{b}	P<0.001

*Abbreviations of dietary treatments are defined in Table 5.1. **Fractional apparent absorption calculated as intake minus faecal excretion and divided by intake. ***Retention calculated as intake minus total excretion (urine and faeces). abcMeans within the same row followed by the same lower case letter are not significantly different.

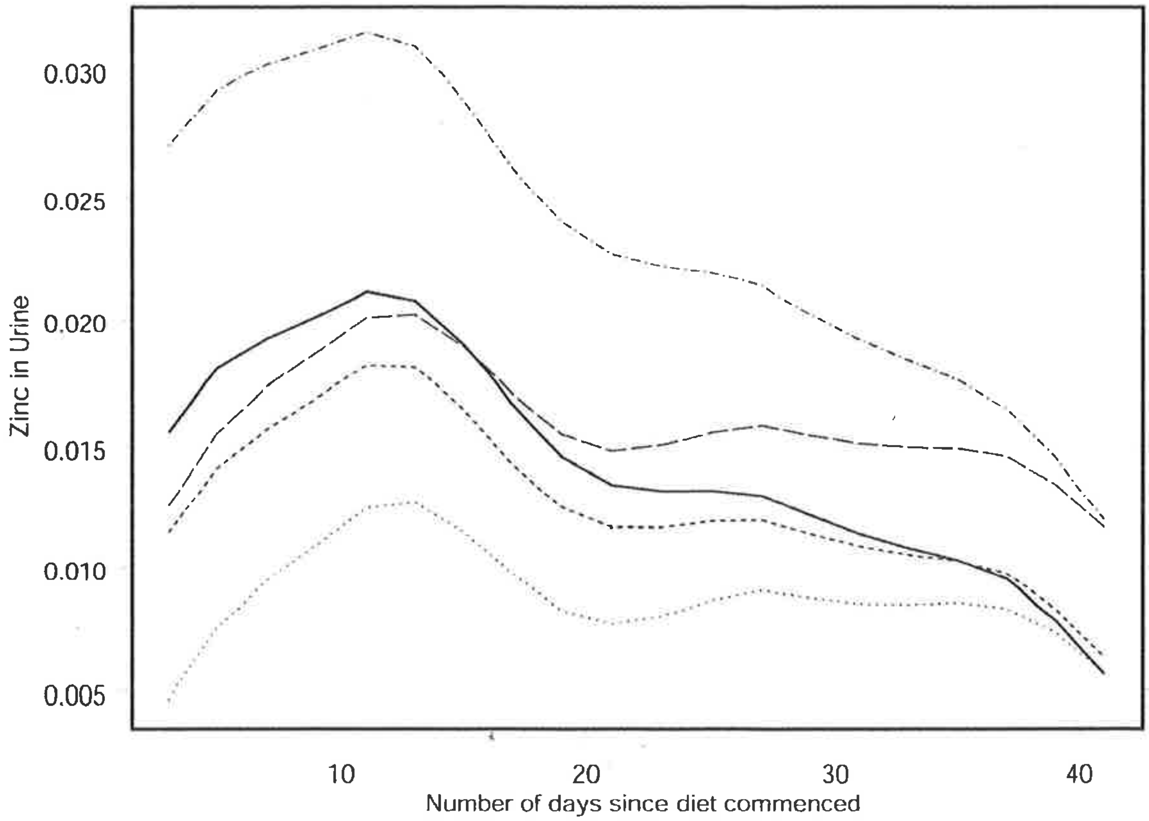


Figure 5.5 The final linear model of urinary zinc excretion (mg/day) by rats fed dietary treatments over a period of 42 days. The dietary treatments (abbreviations defined in Figure 5.2) include ORG (_ _ _ _ _), ORG+PHYT (______), CONV (.......), CONV+Zn (_ _ _ _ _) and SYN (_ . _ . _ .).

The fractional apparent absorption of zinc from the feed (ie. the percentage of zinc consumed that was not excreted in the faeces) was significantly lower for the rats fed the SYN diet and significantly higher for those fed the CONV diet compared to all other diets (Table 5.3).

Zinc retention was significantly greater in those rats fed the SYN diet and lower in those fed the CONV diet, compared to the other diets (Table 5.3). There was no significant difference between the rats fed the ORG and ORG+PHYT diets in terms of zinc retention.

The plasma zinc of the rats fed the SYN diet was significantly less than those fed the grain-based diets (Table 5.4). However, there were no significant differences in plasma zinc between the rats fed the grain-based diets. There was no significant difference between any of the dietary treatments in terms of liver and muscle zinc concentrations (Table 5.4).

Table 5.4 Zinc and calcium pools in rats after 6 weeks on the treatment diets* (plasma n=10; other n=5).

	SYN	**ORG**	**ORG+PHYT**	**CONV**	**CONV+Zn**	**F Prob**
Plasma Zn (µg/ml)**	1.10^{a}	1.51^{b}	1.54^{b}	1.40^{b}	1.45^{b}	P<0.001
Liver Zn (mg/kg DW)	78.8	78.2	76.8	87.0	81.4	NS
Muscle Zn (mg/kg DW)	38.4	46.6	43.0	40.0	37.0	NS P=0.058
Femur Zn (mg/kg DW)	185.5^{a}	217.4^{b}	208.4^{ab}	183.6^{a}	225.6^{b}	P=0.001
Plasma Ca (µg/ml)**	115	116	114	113	110	NS
Liver Ca (mg/kg DW)	143.4	122.2	127.8	112.6	135.2	NS
Muscle Ca (mg/kg DW)	386	210	360	221	201	NS
Femur Ca (% DW)	21.22^{b}	19.88^{ab}	19.58^{a}	20.54^{ab}	21.12^{b}	P=0.006

*Abbreviations of dietary treatments are defined in Table 5.1. **Samples from days 30 and 42 only; day 16 samples excluded due to centrifuge malfunction. ^{ab}Means within the same row followed by the same lower case letter are not significantly different. NS indicates that there is no significant difference between the diets (P<0.05).

The zinc concentration of the femurs from the rats fed the SYN and CONV diets was significantly lower than those of the rats fed ORG and CONV+Zn diets (Table 5.4). The rats fed the ORG+PHYT diet were intermediate and not significantly different to the other diets in terms of femur zinc concentration.

5.3.4 Calcium

There was no significant difference between the dietary treatments in terms of calcium intake (Table 5.5). However, the rats fed the SYN diet were excreting significantly less calcium than the rats fed the grain-based diets, between which there was no significant difference (Table 5.5).

The fractional apparent absorption of calcium from the feed was significantly higher in the rats fed the SYN diet and significantly lower in the rats fed the CONV diet, compared to the other three dietary treatments (Table 5.5). Even though the fractional apparent absorption varied between the diets, there was no significant effect of dietary treatment on the calcium retention of the rats (Table 5.5).

There were no differences in plasma, muscle or liver calcium concentrations between the rats fed the different diets (Table 5.4).

The femur calcium concentrations of the rats fed the SYN and CONV+Zn diets were significantly greater than those of the rats fed the ORG+PHYT diet. The rats fed the ORG and CONV diets had intermediate femur calcium concentrations that were not significantly different from the other diets (Table 5.4).

5.3.5 Iron

There was no significant difference in iron intake or total iron excretion (urinary plus faecal) between the dietary treatments (Table 5.6). However, the iron excreted in the urine was significantly greater in rats fed the SYN diet compared with the grain-based diets (Table 5.6). In addition to this, both the fractional apparent absorption and the retention of iron by rats fed the SYN diet was significantly lower than for the rats fed the grain-based diets (Table 5.6).

The percentage of red blood cells in the blood of the rats was significantly lower in those rats fed the SYN diet compared to the rats fed the grain-based diets (Table 5.7). There was no significant difference between the rats fed the grain-based diets in terms of the percentage of red blood cells in the blood.

Table 5.5 Mean calcium balances over 6 weeks (n=100; 5 rats, 20 measurements) in rats fed the treatment diets*.

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
Ca Intake (mg/day)	96.91	103.94	104.44	99.03	99.25	NS
Total Ca Excreted (mg/day)	13.78^{a}	23.40^{b}	23.67^{b}	25.40^{b}	23.28^{b}	P<0.001
Ca in Urine (mg/day)	2.24	2.50	3.59	2.75	2.91	NS
Fractional Apparent Absorption of Ca** (%)	88.84^{c}	80.34^{b}	81.14^{b}	77.77^{a}	80.40^{b}	P<0.001
Ca Retention*** (mg/day)	83.18	80.58	81.12	73.43	75.95	NS

*Abbreviations of dietary treatments are defined in Table 5.1. **Fractional apparent absorption calculated as intake minus faecal excretion and divided by intake. ***Retention calculated as intake minus total excretion (urine and faeces). ^{abc}Means within the same row followed by the same lower case letter are not significantly different. NS indicates that there is no significant difference between the diets (P<0.05).

Table 5.6 Mean iron balances over 6 weeks (n=100; 5 rats, 20 measurements) in rats fed the treatment diets[*].

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
Fe Intake (mg/day)	0.678	0.649	0.663	0.689	0.671	NS
Total Fe Excreted (mg/day)	0.482	0.401	0.383	0.421	0.409	NS
Fe in Urine (mg/day)	0.017^{b}	0.003^{a}	0.003^{a}	0.006^{a}	0.005^{a}	P<0.001
Fractional Apparent Absorption of Fe[**] (%)	31.28^{a}	40.24^{b}	43.15^{b}	40.24^{b}	40.98^{b}	P<0.001
Fe Retention[***] (mg/day)	0.204^{a}	0.259^{b}	0.295^{b}	0.274^{b}	0.269^{b}	P<0.001

[*] Abbreviations of dietary treatments are defined in Table 5.1. [**] Fractional apparent absorption calculated as intake minus faecal excretion and divided by intake. [***] Retention calculated as intake minus total excretion (urine and faeces). ^{ab}Means within the same row followed by the same lower case letter are not significantly different. NS indicates that there is no significant difference between the diets (P<0.05).

Table 5.7 Iron pools in rats after 6 weeks on the treatment diets[*] (plasma n=10; other n=5).

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
% Red Blood Cell[**] (Haematocrit)	38.4^{a}	44.3^{b}	44.0^{b}	43.9^{b}	44.4^{b}	P=0.005
Plasma Fe (µg/ml)[***]	4.36^{a}	8.06^{b}	7.49^{ab}	6.31^{ab}	6.42^{ab}	P=0.040
ORG(+), CONV(+)		7.77		6.36		NS P=0.086
Liver Fe (mg/kg DW)	144^{a}	241^{ab}	173^{ab}	178^{ab}	244^{b}	P=0.041
Muscle Fe (mg/kg DW)	18.2^{a}	29.4^{b}	25.4^{b}	25.3^{b}	26.8^{b}	P<0.001
Femur Fe (mg/kg DW)	33.1^{a}	59.4^{b}	52.9^{b}	55.2^{b}	57.7^{b}	P=0.001

[*] Abbreviations of dietary treatments are defined in Table 5.1. [**] Samples from day 42 only. [***] Samples from days 30 and 42 only; day 16 samples excluded due to centrifuge malfunction. [ab] Means within the same row followed by the same lower case letter are not significantly different.

The plasma iron of the rats fed the SYN diet was significantly less than for those fed the ORG and ORG+PHYT diets (Table 5.7). The rats fed CONV and CONV+Zn diets had intermediate plasma iron concentrations, which were not significantly different to the other diets. However, there was a non-significant (P=0.086) trend towards the rats fed ORG(+) diets having greater plasma iron than the rats fed CONV(+) diets (Table 5.7).

The liver iron concentrations of the rats fed the SYN diet were significantly lower than those of the rats fed the CONV+Zn diet (Table 5.7). The rats fed the other diets were intermediate in liver iron concentration and not significantly different from either the SYN or CONV+Zn dietary treatments

The rats fed the SYN diet had significantly lower muscle iron concentrations than the rats fed the grain-based diets (Table 5.7). There was no significant difference in muscle iron concentration between the grain-based dietary treatments.

The iron concentration of the femur was significantly lower for the rats fed the SYN diet compared with those fed the grain-based diets (Table 5.7). There was no significant difference in femur iron concentration between the rats fed the grain-based diets.

5.3.6 Breath Analysis

On day 30, with the dietary treatment as the substrate, the breath hydrogen concentrations of rats fed the ORG(+) diets was significantly greater than that of rats fed the CONV(+) diets (Figure 5.6). The rats fed the SYN diet were intermediate and did not significantly differ from the other diets in hydrogen production but produced significantly greater methane than the grain-based diets (Figure 5.6) as determined by breath concentration.

Using lactulose as a substrate (Day 39), there were no significant differences in either hydrogen or methane production between the grouped dietary treatments at any measurement time after the lactulose meal (results 180 minutes after the meal are shown in Figure 5.7), except in hydrogen production 60 minutes after the meal (this result is discussed in Appendix 11).

Other hydrogen and methane data are presented and discussed in Appendix 11.

The ^{13}CO2 production of the rats on the various treatment diets is included in Appendix 11. There was no significant difference between the diets in peak height, time of peak height (T_{max}) or T(1/2) (Table 5.8). However, both the oro-caecal transit time (OTT) and small intestinal transit time (SITT) differed significantly between the dietary treatments (Table 5.8). The ORG fed rats recorded a significantly greater OTT and SITT than those fed the SYN diet. The rats fed CONV, CONV+Zn and ORG+PHYT diets were intermediate and statistically similar to both ORG and SYN treatments.

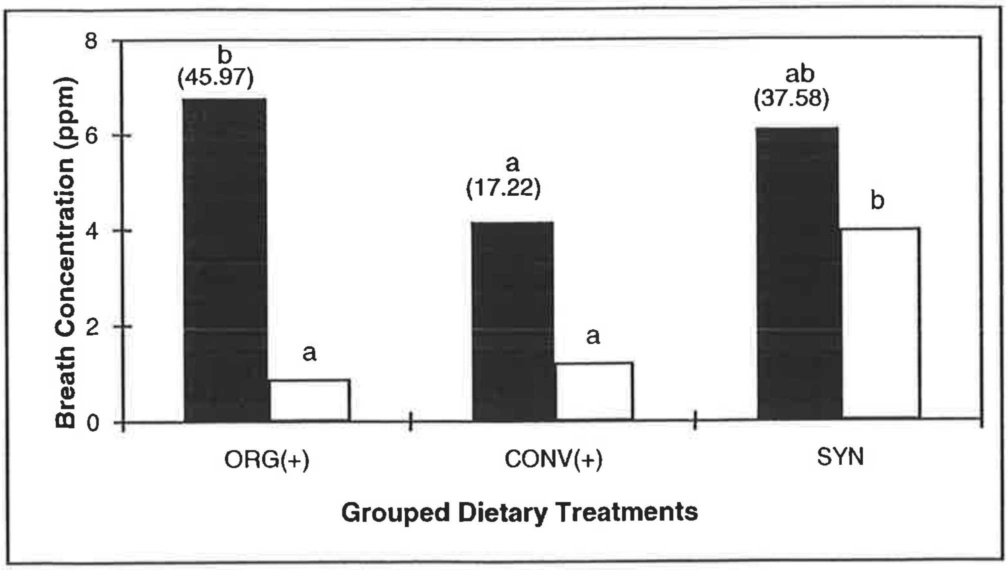


Figure 5.6 Breath hydrogen (■) and methane (□) concentrations of rats fed diets based on organically (ORG(+)) or conventionally (CONV(+)) grown grain or the AIN-93G formulation (SYN) for 30 days with the substrate for the test being the treatment diet. The hydrogen data has been square root transformed. Values in parentheses are back transformed for interpretive purposes. Treatments within each gas type with the same lower case letter are not significantly different (P<0.05).

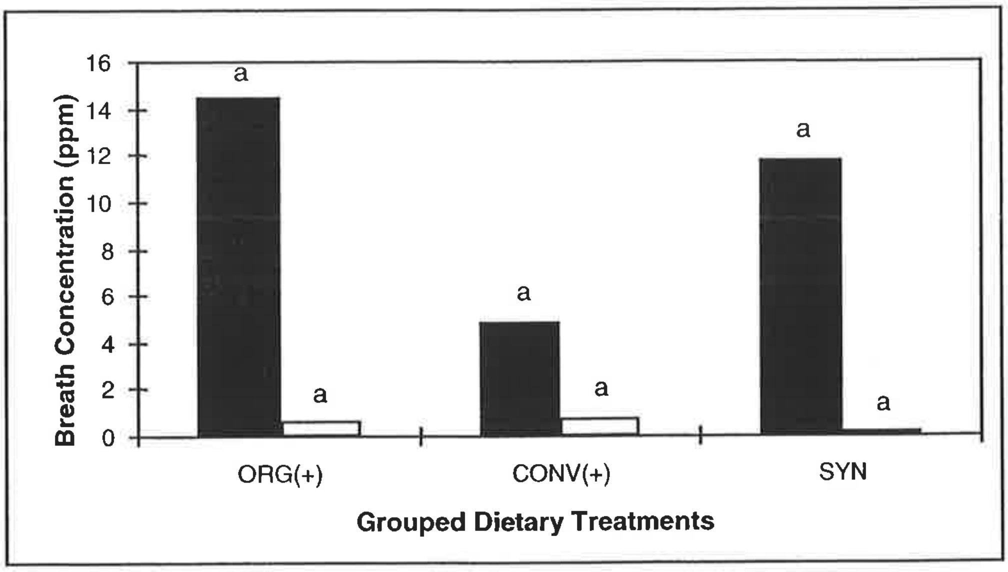


Figure 5.7 Breath hydrogen (■) and methane (□) concentrations of rats fed diets based on organically (ORG(+)) or conventionally (CONV(+)) grown grain or the AIN-93G formulation (SYN) with the substrate being lactulose administered after a 12 hour fast. Measurements were taken 180 mins after the lactulose meal. Treatments within the same gas type with the same lower case letter are not significantly different (P<0.05).

Table 5.8 Characteristics of the breath ^{13}C and H curves of rats fed different diets* (n=5) as an indication of the movement of substrate through the gastrointestinal tract.

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
^{13}C Peak height (DoB)	85.15	75.04	76.83	95.39	76.80	NS
T_{max} (min) (time of ^{13}C peak height)	18.48	17.62	19.75	16.49	20.5	NS
$T_{(1/2)}$ (min) (gastric half emptying time)	44.07	38.99	44.95	37.39	44.67	NS
OTT (min) (time to reach caecum)	27.13^{a}	110.05^{b}	88.01^{ab}	96.3^{ab}	103.11^{ab}	P=0.028
SITT (min) (estimated rate of passage in small intestine)	-16.94^{a**}	71.06^{b}	43.06^{ab}	58.91^{ab}	58.44^{ab}	P=0.019

*Abbreviations of dietary treatments are defined in Table 5.1. **A negative SITT indicates that the first of the substrate has reached the caecum before half of the substrate has left the stomach. ^{ab}Means within the same row followed by the same lower case letter are not significantly different. NS indicates that there is no significant difference between the diets (P<0.05).

5.4 Discussion

5.4.1 Feed Intake and Growth

A reduction in feed consumption is usually associated with severe zinc deficiency (Williams and Mills, 1970; Davies and Nightingale, 1975) due to zinc deficiency causing loss of appetite. The fact that there was no difference in feed consumption among the dietary treatments in the current study signifies that none of the rats are overtly zinc deficient. This verdict is backed up by the fact that there is no difference in growth rate, femur or body mass (Appendix 10 and Table 5.2) between the rats fed the two levels of zinc. Growth is compromised when zinc deficiency is severe (Forbes and Yohe, 1960; Williams and Mills, 1970; Brown *et al.*, 1978; O'Leary *et al.*, 1979). Based on critical zinc requirements for growth of young rats as calculated by Forbes and Yohe (1960) (11.6-17.8 mg zinc/kg diet, depending on the protein source), the rats in the current study were not expected to be overtly zinc deficient. However, marginal zinc deficiency (ie. not manifested in growth related responses) is certainly a possibility. More subtle responses due to marginal zinc deficiency could include gut function, immune status and metabolic activities of zinc dependent enzymes.

The reduction in feed consumption that was observed following blood sampling (Figure 5.3) is a common response by rats to stress (Weninger *et al.*, 1999). The stress of restraint and extra handling and the trauma of the tail vein procedure could account for this. The fact that this reduction was significantly greater in those rats fed the ORG+PHYT diet than any of the other diets, suggests that these rats were under greater stress or reacting differently to stress than those in the other dietary treatments. A possible mechanism is a temporary reduction in small intestinal transit time (shown by Muraoka *et al.* (1998) to occur in response to restraint stress), resulting in a full stomach and loss of appetite. The femur length and mass of the ORG+PHYT fed rats tended to be lower than those of rats fed the other diets (Table 5.2). These factors hint that the ORG+PHYT fed rats may be under long-term dietary-induced stress, which has also manifested in lower tolerance to the external stress of the restraint involved in blood sampling.

The only difference between the ORG and ORG+PHYT diets was the addition of sodium phytate to the latter diet (the sodium was balanced with that of the other diets). The rats fed the ORG diet did not have reduced femur mass and neither did those rats fed the CONV diet, which had the same level of phytate as the ORG+PHYT diet. This points to a difference between the synthetic phytate and the endogenous phytate of the grain. An obvious difference is the cations within the salt. The synthetic phytate was

Increases in faecal FW, DW and water content (as observed here in the grain-based diets compared with the SYN diet) have been previously shown to be responses to starch malabsorption (Scheppach *et al.*, 1988). This implies that the grain-based diets result in a greater malabsorption of starch, and possibly other carbohydrate types such as non-starch polysaccharides and non-digestible oligosaccharides, than the SYN diet.

An increase in transit time (concurrent with increases in faecal FW, DW and water content) has also been shown to be associated with starch malabsorption (Scheppach *et al.*, 1988). This supports the hypothesis that the grain-based diets in the current study have greater malabsorption, because the rats fed the SYN diet tended to have a faster transit time than those fed the grain-based diets and significantly faster than the rats fed the ORG diet (Table 5.8). However, the rapidity of the transit time for the rats fed the SYN diet could also result in some of the carbohydrate escaping digestion in the small intestine, and therefore malabsorption. This possibility was supported by the breath hydrogen content (an indicator of microbial colonic fermentation and hence malabsorption) of the rats, which indicated that there was no difference in malabsorption between the SYN and grain-based dietary treatments except at day 42, which may have been affected by fasting stress (discussed in Appendix 11). This conflicting evidence suggests that the effect of malabsorption on faecal FW, DW and water content may be due to the presence of a specific type of indigestible carbohydrate, whereas hydrogen production in the colon is not substrate specific.

In terms of the grain-based diets, the ORG (but not the ORG+PHYT) fed rats expressed a significantly greater rate of increase in FW faeces production over the 6 weeks of the experiment in comparison to the other dietary treatments (Figure 5.4). Therefore, the ORG fed rats are behaving quite differently to the other dietary treatments. The lower phytate level is implicated because in every other way, the ORG and ORG+PHYT diets are identical. The greater rate of increase in faecal production (FW) by rats fed the ORG diet may be an indication of an adaptation over the time of the experiment to either lower (ORG diet) or higher phytate (ORG+PHYT, CONV and CONV+Zn diets) in the diet. However, there is no evidence in the literature of phytate altering faecal water content or volume.

Even if lower phytate is associated with the greater faecal production (FW and DW) of the rats fed the ORG diet, the greater faecal production and faecal water contents of the rats fed the ORG(+) diets in comparison to those fed the CONV(+) diets points to the involvement of some other factor that was inherently different between the organically and conventionally grown grain that was not elucidated, and therefore not accounted for in the balancing of the diets. Researchers have shown that changes in the carbohydrate fractions have resulted in changes in faecal production (Tsai *et al.*, 1983).

It is therefore possible that this unknown factor is associated with the carbohydrate or fibre fractions of the diet and these did differ between the 'organic' and 'conventional' grain used to formulate the diets (Table 5.9).

Table 5.9 Compositional differences between the organically and conventionally grown wheat samples used to formulate the grain-based diets: ORG, ORG+PHYT, CONV and CONV+Zn.

	Organic	**Conventional**
Available Carbohydrate (%)	68.9	79.2
Total Starch (%)	46.4	49.7
Fibre (%) (NDF)	36.7	42.7
Hemicellulose (%)*	33.0	39.3
Cellulose + Lignin (%) (ADF)	3.8	3.3

*Hemicellulose was calculated as the difference between NDF and ADF values.

The starch and fibre contents were greater in the conventionally than the organically grown grain used in formulating the diets (Table 5.9). This difference in content between the grains suggests that there may also have been differences in the digestibility of these grains. It is possible, for example, that the organically grown grain used in the diets had a greater content of resistant starch (that is, starch resistant to digestion in the small intestine) than the conventionally grown grain, even though the total starch content was greater in the conventionally grown grain. If this was so, a greater amount of resistant starch in the ORG(+) diets may have resulted in greater faecal production and water content. It has been shown that the presence of resistant starch is associated with greater faecal water content (Scheppach *et al.*, 1988) and greater dry weight faecal excretion (Scheppach *et al.*, 1988; Hylla *et al.*, 1998). Both of these were observed in the current study (Table 5.1).

There is less available (that is, digestible) carbohydrate in the organically grown grain than the conventionally grown grain used in the diets (Table 5.9). This may mean that there is greater non-absorbable but fermentable carbohydrates in the organically grown grain than the conventionally grown grain. Lactulose is an example of a non-absorbable and readily fermentable carbohydrate and it has been shown that lactulose increases the faecal water content (Terada *et al.*, 1992). Similarly, polysaccharides present in soy have been shown to increase the FW faecal excretion by increasing the faecal water content (Tsai *et al.*, 1983).

Whether the ORG(+) diets resulted in greater malabsorption of starch or other carbohydrates, could only be confidently determined by analysing digesta for these components. However, the breath hydrogen content of the rats fed the ORG(+) diets was greater than those fed the CONV(+) diets when the substrate was the dietary treatment (Figure 5.6) and no different when the substrate was lactulose (ie. non-absorbable) (Figure 5.7). This supports that malabsorption was greater in the ORG(+) diets and that the difference in hydrogen production between the rats fed the ORG(+) and CONV(+) diets was not due to a difference in the microbial populations in the colon, but because of a difference in the supply of non-digestible carbohydrates by the grains used in the diet formulations.

5.4.3 Nutrient Balance

Synthetic Diet

Although the rats fed the SYN diet appeared to perform similarly, in terms of growth parameters, to the rats fed the grain-based diets, the SYN fed rats had low iron and zinc status in blood plasma and bone, and low iron status in soft tissue. Low iron and zinc indices are symptomatic of anaemia.

The faster transit time (Table 5.8) of the SYN diet would have resulted in less opportunities for nutrient absorption, but would probably not have had an effect great enough to cause anaemia. The puzzling result is that the rats fed the SYN diet have a significantly higher zinc intake than the rats fed the grain-based diets and a correspondingly higher calculated retention of zinc (Table 5.3). This unexpected result has not been reported in the literature. In fact, researchers report reduced zinc retention in correlation with lower plasma or bone zinc (House *et al*, 1982; Hallmans *et al.*, 1989).

The major modification of the SYN diet from the AIN-93G diet was the use of EDTA-washed casein. This washing was done in order to reduce the amount of zinc added through the casein but may have had the unanticipated side-effect of either removing zinc and iron from the diet through residual EDTA or by increasing the binding capacity of the casein for other cations, for example iron. Both iron and zinc are chelated by EDTA, but it has been shown, at least for iron EDTA, that the chelate complex dissociates within the gastrointestinal tract, releasing the iron in a readily absorbable form (Heimbach *et al.*, 2000). In fact, iron EDTA is used for iron fortification of foods and has been demonstrated to be safe for consumption when a diet contains up to 5 percent EDTA (Heimbach *et al.*, 2000). For this reason, chelation by residual EDTA is not likely to have caused the anaemic iron levels in the rats.

The increased binding capacity of the casein is much more likely to have caused the anaemic iron levels. Iron is oxidised and strongly bound by casein (Emery, 1992). The resulting complex does not dissociate in response to pH changes (pH 2.5-6.8 tested) (Gaucheron *et al.*, 1996; Gaucheron *et al.*, 1997), whereas the stability of zinc-casein complexes is pH dependent (dissociation below pH 5) (Harzer and Kauer, 1982; Gaucheron *et al.*, 1997). The presence of inorganic phosphate also precluded the dissociation of the iron-casein complex (Gaucheron *et al.*, 1996). Even though the pH of the gut may be lower than 2.5, the iron-casein complex is unlikely to dissociate under biological acidification in the gut because the iron is thought to be bound to the phosphoseryl residues of the casein by coordinate bonds rather than the ionic bonds of other cation-casein complexes (Gaucheron *et al.*, 1996). This would result in the iron being unavailable from the iron-casein complex, resulting in a lower fractional apparent absorption of iron, as was observed in the current study (Table 5.6). It would manifest in the lower haematocrit readings and lower blood plasma and femur iron concentrations also observed in the current study (Table 5.7). Although the anaemiasymptoms (low plasma iron and zinc and low liver, muscle and bone iron), thought to have developed due to this complexation, render the SYN diet a poor representative of previous studies, it is a vivid illustration of the complexities involved with bioavailability and diet formulation. It is important, therefore, to take care in modifications of the current recommendation for diet and feed formulations and the use of the Recommended Daily Intake (RDI).

Grain-Based Diets

Among the grain-based diets, the lower excretion of zinc corresponding with the lower zinc intake and retention of rats fed the CONV diet (Table 5.3) suggests that the rats have a marginal zinc status and that they are absorbing as much zinc as possible from the diet. This was reflected in a greater fractional apparent absorption of zinc by the CONV fed rats (Table 5.3). Other researchers have also found in both rats (Forbes and Yohe, 1960; Weigand and Kirchgessner, 1980) and humans (Wada *et al.*, 1985) that lower dietary zinc levels result in reduced zinc excretion (as a percentage of intake) and therefore, greater fractional absorption. No suggestions have been made by researchers as to the mechanism involved in this response.

The zinc retention, as calculated in a balance study such as this one, may not represent the actual amount of zinc absorbed into the bloodstream, and hence available to tissues other than the gut, because not all zinc retained within the body is absorbed into the bloodstream. Some zinc is retained in the gut within the mucosal lining[1] in association

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[1] Although zinc retained in the mucosal lining of the gut can be considered to be absorption in the broader

with binding proteins, hence facilitating nutrient absorption. The true zinc absorption (into the bloodstream) of the rats could only be determined through the use of zinc isotopes within the diets, which could be extremely difficult within the context of the current investigation. Although grain can be naturally laced with isotopic zinc through the addition of fertiliser containing the desired isotope, this would be prohibited within an organic system due to the requirement of natural fertilisers.

Further to the discussion on the calculated zinc retention and absorption, the zinc present in faeces is not only the zinc that has not been absorbed as the substrate passes through the gastrointestinal tract. Faecal zinc also includes endogenous secretions of zinc in pancreatic juices and bile and the loss of zinc when mucosal cells are sloughed. Therefore, it is possible in the current study, that the apparent adaptation of the CONV fed rats to the lower zinc diet may be achieved through the conservation of endogenous zinc secretions rather than enhanced absorption of zinc. Researchers have found that with marginal zinc intakes, the exogenous excretion of zinc is unchanged (as a percentage of intake) (Sian *et al.*, 1996) but that there is less endogenous zinc excreted (Lee *et al.*, 1993; Sian *et al.*, 1996). However, because zinc isotopes were not used in the current study, no distinction was possible between the exogenous and endogenous zinc excreted.

Although the nutrient balance data hint at marginal zinc deficiency in the rats fed the CONV diet, the blood plasma results showed no significant difference between the grain-based diets in terms of zinc status (Table 5.4). However, it has been argued that plasma zinc is an inappropriate index for zinc deficiency, particularly under marginal zinc status as in the current study (King, 1990; Wood, 2000), because the blood is the vehicle through which zinc is mobilised from the bodily stores to the places where it is required. Plasma zinc status remains in use as an index of zinc deficiency, though, because no superior non-terminal index has been identified to date.

One marker suggested to have some limited value in assessing marginal zinc status is metallothionein (King, 1990; Wood, 2000). Metallothionein is an intracellular zinc storage protein and is associated with zinc metabolism because it is up-regulated at excess levels of dietary zinc, reducing zinc absorption and down-regulated at low levels of dietary zinc to increase zinc absorption. However, the dietary zinc levels in the current experiment (15-35 mg/kg) are not high enough to cause metallothionein induction which has only been detected when dietary zinc (shown using synthetic zinc) is 400 mg/kg or greater (Tran *et al.*, 1999). Likewise, it has been shown that for diets

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sense, it is not systemic absorption.

with zinc as low as 10 mg/kg, metallothionein concentrations in the gut are not different to when the dietary zinc levels are 100 mg/kg (Tran *et al.*, 1999) and therefore it is unlikely that metallothionein is involved in the greater fractional apparent absorption of zinc associated with the CONV diet. However, the increased fractional apparent absorption of zinc by rats fed the CONV diet is an important finding because it indicates that the CONV fed rats have had to increase their absorptive capacity by some means, in order to maintain zinc status. This implies that the CONV diet is probably marginally deficient in zinc.

The femur zinc concentrations were significantly lower in the CONV fed rats (Table 5.4), inferring that these rats are at least zinc restricted and possibly even marginally zinc deficient. Particularly in the case of marginal zinc status (where a growth response is not observed), bone zinc is an appropriate index of zinc bioavailability (Baker and Ammerman, 1995) and is widely used in experimental work. Zhou *et al.* (1993) found in comparing rats fed either 6 mg or 12 mg (pair fed) zinc per kg diet, that although there was little difference between the two in zinc concentrations in muscle and liver over 5 weeks, it only took two weeks until the rats fed the higher zinc diet had significantly greater zinc concentration in the bone (230-279 mg/kg over weeks 2-5, compared with 139-156 mg/kg for the zinc restricted diet). Zhou *et al.* (1993) showed using radio-labelling that the rats fed the restricted diet experienced a removal of zinc from the bone, and therefore probably marginal zinc deficiency, whereas the pair fed rats, although also experiencing a reduction of zinc concentration (compared with a baseline of 300 mg/kg), had zinc deposited in their bones. The bone zinc values (for the CONV and the higher zinc diets) in the current study all lie between the values reported by Zhou *et al.* (1993) for zinc restricted and pair fed rats. It is possible therefore, that all rats in the current study are marginally zinc deficient, but that the ones fed the CONV diet are significantly more so.

In the current study, like the findings reported in the study by Zhou *et al.* (1993), both the low (CONV) and the higher zinc dietary treatments had similar muscle and liver zinc concentrations. This occurs because under marginal conditions, zinc is likely to be mobilised from the bone and moved to the metabolically active parts of the body, for example the muscle and liver.

Although there seems to be no effect on zinc bioavailability in the current study as a result of the addition of phytate (except perhaps in the femur zinc concentration), this may be due to the background phytate present within all the grain-based diets. The effect of phytate in reducing zinc absorption by increasing faecal zinc excretion has been clearly elucidated by other researchers (Davies and Nightingale, 1975; House *et al.*, 1982; Flanagan, 1984; Saha *et al.*, 1994). Although many of the phytate-zinc studies involve the addition of synthetic phytate (as in the ORG+PHYT diet), intrinsic phytate in diets (as in all grain-based diets in the current study) has been shown to have similar effects on zinc bioavailability (Morris and Ellis, 1980; Zhou *et al*, 1992; Saha *et al.*, 1994).

There is some evidence that the molar ratio of phytate:zinc in a diet is a predictor of the zinc bioavailability of that diet. The greater the phytate:zinc molar ratio, the smaller the weight gain of rats and the lower the bone zinc content (Morris and Ellis, 1980; Forbes *et al.*, 1984; Fordyce *et al.*, 1987). The phytate:zinc molar ratios of the diets in the current study are shown in Table 5.10. Based on the results of other researchers (Morris and Ellis, 1980; Forbes *et al.*, 1984; Fordyce *et al.*, 1987), there is enough range in the phytate:zinc molar ratios of the diets in the current study to obtain a growth response to zinc as well as differences in bone zinc. The fact that no growth response was obtained over the 6 weeks of the experiment (Table 5.2), may attest to the greater importance of the absolute zinc concentration in the diet than the phytate:zinc molar ratio.

A further extension of the molar ratio theory is the inclusion of calcium. Calcium has been shown to have a negative effect on zinc bioavailability (Forbes *et al.*, 1984), possibly through competition for common absorption sites in the small intestine. Fordyce *et al.* (1987) found that zinc bioavailability was poor (with a significant effect on growth and bone zinc) when the phytate x calcium:zinc molar ratio was greater than 3.5. The only grain-based diet in the current study with the respective ratio lower than 3.5 was the ORG diet (Table 5.10). However, there was no difference in growth response between the rats fed the ORG and the CONV (with the highest ratio of 6.6) diets but a difference corresponding to the phytate x calcium:zinc molar ratio was apparent in the femur zinc concentration. This is probably due to the marginal zinc status of the rats in the current study compared with lower zinc levels used in the study by Fordyce *et al.* (1987).

Because the mechanism of the calcium and zinc interaction is thought to be that these nutrients compete for common absorption sites, the interaction between these two nutrients is also reciprocal. High dietary zinc (roughly 10 times the normal intake) has been shown to reduce plasma calcium and the intestinal absorption of calcium but only when calcium intake is low (Spencer *et al.*, 1987). In the current study, calcium intakes were sufficient and, therefore, there was no effect of dietary zinc on plasma calcium (Table 5.4). In addition, the dietary zinc intake in the current study was not greater than that recommended in the AIN-93G diet and therefore none of the dietary treatments have high zinc as in the study by Spencer *et al.* (1987).

Table 5.10 Molar ratios of phytate, zinc and calcium in the treatment diets*.

	Phytate:Zn Molar Ratio	Phytate x Ca:Zn Molar Ratio
SYN	No Phytate	No Phytate
ORG	18.7	2.3
ORG+PHYT	32.7	4.1
CONV	53.3	6.6
CONV+Zn	32.7	4.1

*Abbreviations of dietary treatments are defined in Table 5.1.

Even though there was no difference between the dietary treatments in terms of plasma calcium, the femur calcium concentrations were greater in rats fed the SYN and the CONV+Zn diets than the other dietary treatments. These two diets were the only ones in which synthetic zinc was added (compared with the ORG and ORG+PHYT diets which had the same zinc content as the CONV+Zn diet). It is possible that the endogenous zinc in the grain is present as metalloprotein or phytate complexes and these have different absorption pathways to that of zinc cations, which possibly have to compete with calcium for absorption sites. Although zinc retention is similar between the CONV+Zn, ORG and ORG+PHYT dietary treatments, the calcium in the bones is greater (either by increased calcium deposition or decreased calcium resorption) when the zinc is present as a cation (CONV+Zn diet) rather than a complex (for example, metalloprotein complexes, which are absorbed without being broken down). This may mean that uncomplexed zinc is required within enzyme systems associated with calcium deposition.

It has been shown that with sufficient calcium, zinc deficiency results in increased levels of parathyroid hormone (Roth and Kirchgessner, 1989), which is normally induced by low levels of plasma calcium and acts to maintain plasma calcium levels. One way in which parathyroid hormone achieves this is by inducing the production of the active form of vitamin D_3 (calcitriol), which increases the absorption of calcium from the small intestine. Roth and Kirchgessner (1989) showed that zinc deficiency does induce the production of calcitriol. If calcitriol also increases the absorption of zinc from the small intestine, parathyroid hormone could also be central to maintaining plasma zinc levels. This then, is hypothesised here to be the possible mechanism by which the rats fed the CONV diet had lower zinc intake but no difference in plasma zinc and a higher fractional apparent absorption of zinc than the other diets.

It is important to note, however, that although fractional apparent absorption of zinc is increased in the rats fed the CONV diet, fractional apparent absorption of calcium is reduced, compared with the other grain-based diets. This suggests that although calcitriol may increase the intestinal absorption of both calcium and zinc, the plasma concentrations of these nutrients may also play a significant role in the regulation of absorption.

Another way in which parathyroid hormone maintains plasma calcium, and possibly also plasma zinc (lower femur zinc concentration in the CONV treatment), is to activate bone resorption. In support of the effect of zinc deficiency on increasing the production of parathyroid hormone, it has been shown *in vitro* for low zinc levels that as the addition of zinc increases, bone resorption is reduced (Holloway *et al.*, 1996). However, it has also been shown *in vivo* that for high levels of dietary zinc (greater than 100 mg/kg diet), increased zinc is associated with increased bone resorption when the active form of vitamin D_3 is also supplemented (Soares *et al.*, 1987). It appears therefore, that zinc is important in inhibiting bone resorption but that high zinc, combined with nutrient interactions, could be detrimental to bone strength. Unfortunately, it is not possible to determine from the measurements made in the current study if bone resorption is an important factor in determining the femur calcium concentration.

The femur calcium concentrations tended to be lower for the rats fed the diet where synthetic phytate was added (ORG+PHYT diet), particularly compared with those where synthetic zinc was added (CONV+Zn and SYN diets) (Table 5.4). This suggests that the synthetic phytate is binding calcium more strongly than the endogenous phytate, probably due to the fact that endogenous phytate is already strongly complexed whereas sodium phytate is not (section 5.4.1).

The similar iron intakes, iron excretion, fractional apparent absorption of iron and iron retention of the rats fed the grain-based diets authenticate the similar haematocrit, blood plasma iron, liver iron, muscle iron and femur iron contents of the grain-based dietary treatments. This is of interest in the context of the reported zinc and iron antagonistic interaction. There are a number of studies (summarised by Whittaker, 1998) showing iron supplementation inhibiting zinc absorption and that when the iron and zinc are administered within a meal, there is no antagonism on zinc absorption. However, there are fewer studies investigating the effect of zinc on iron absorption. It has been shown that although zinc supplementation reduces the absorption of an iron supplement, it does not do so when both are administered within a meal (Rossander-Hulten *et al.*, 1991). Although the iron contents of the diets in the current study were all the same, the CONV diet had lower zinc than the other grain-based diets. Although this difference in zinc may have elicited a response in iron metabolism if the zinc and iron were given as supplements separate from meals, the zinc and iron were present within the diets consumed by the rats, and therefore the fact that the different zinc levels had no effect on iron metabolism, is consistent with the findings of Rossander-Hulten *et al.* (1991).

The rats fed the ORG(+) diets had a greater reduction in urinary zinc excretion towards the end of the experiment than the rats fed the CONV(+) diets (Figure 5.5). There was also a non-significant trend (P=0.086) towards the ORG(+) fed rats having greater plasma iron concentrations than the CONV(+) fed rats (Table 5.7). This latter observation may be due to a slightly greater addition of synthetic iron due to the organically grown grain used in this experiment containing slightly less iron than the conventionally grown grain. However, the presence of these differences between the ORG(+) and CONV(+) diets indicates again (section 5.4.2) that there were undefined differences between the organically and conventionally grown grain used in this experiment and that these differences also had some effect on zinc and iron homeostasis.

Phytase, and possibly alkaline phosphatase (Davies and Flett, 1978), are the enzymes involved in the digestion of phytate. Wheat has one of the highest phytase activities (Eeckhout and DePaepe, 1994). Although the level of phytase activity in the wheat was not determined in the current study, the level of phytase activity within a diet has shown to be of importance in phosphorus bioavailability in pigs (Pointillart *et al.*, 1987) and humans (Sandberg and Andersson, 1988).

In fact, without dietary phytase, humans digest very little phytate (Sandberg and Andersson, 1988). This indicates low levels of intestinal phytase. However, rats have around 30 times the intestinal phytase activity of humans (Iqbal *et al.*, 1994). Because of this characteristic, rats are not the most ideal model for humans in terms of phytate metabolism. However, other small mammals also have greater intestinal phytase activities than humans (from Cooper and Gowing (1983) and Iqbal *et al.* (1994)). Care must be taken, therefore, when extrapolating the current results to human applications.

It has also been found that in rats, adaptation to dietary phytate results in greater activity of intestinal phytase (Cooper and Gowing, 1983; Lopez *et al.*, 2000). Cooper and Gowing (1983) found that this adaptation in the rat *in vitro* was greater than that of other small mammals with lower intestinal phytase activities. This suggests that adaptation to phytate through increasing phytase activity may be quite low in humans, as was found by Brune *et al.* (1989). Therefore, the effect of phytate within the dietary treatments of the current study (differences between the ORG, ORG+PHYT and CONV+Zn diets) may be more pronounced in human subjects.

Phytase activity has also been shown to be reduced in rats when their zinc status is low (Davies and Flett, 1978; Cooper and Gowing, 1983). The corresponding reduction in phytate digestion may further reduce available zinc if dietary zinc has been bound by phytate. This was probably not a contributing factor to the comparatively low zinc retention of the rats fed the CONV diet in the current study, however, because fractional apparent absorption of zinc was greater for the rats fed the CONV diet.

It was noted earlier in this discussion that the added synthetic phytate (sodium phytate) present in the ORG+PHYT diet appears to have elicited a different response in some cases (for example, in growth and in the iron concentrations within liver, muscle and bone) than the CONV+Zn diet which had the same endogenous phytate content plus the same zinc content. Part of the key to this difference may lie in the intestinal phytase activity of the rats. Lopez *et al.* (2000) found that rats fed sodium phytate had most of the phytase activity in the duodenum and jejunum (the upper small intestine), whereas rats fed the same phytate level but contained endogenously in wheat bran, had phytase activity in the ileum only (the lower small intestine).

In addition to this, because endogenous phytate is present within protein bodies (Batten and Lott, 1986; Batten *et al.*, 1994; Wada and Lott, 1997), it is less accessible to phytase than synthetic phytate is.

5.5 Summary

- The first aim of this part of the study was to determine if the combined effect of lower zinc and higher phytate in the conventionally, compared to organically, grown wheat will induce zinc deficiency in rapidly growing rats.

There was no significant effect of the lower zinc and higher phytate of the conventionally grown grain on rat growth, food intake or excretion and, therefore, no overt zinc deficiency. It was found, however, that marginal zinc status, indicated by lower zinc retention, greater fractional apparent absorption of zinc and lower bone zinc concentration, was induced in weanling rats fed the diet based on conventionally grown grain and not supplemented with zinc. The same degree of marginal zinc deficiency was averted by supplementing with zinc to the level of the organically grown grain and also when rats were fed diets based on organically grown grain. It was difficult to conclusively determine the contribution of the lower phytate in the organically grown grain to the improved zinc status because the interaction between zinc and phytate appeared to depend on the form of the phytate (synthetic or endogenous) in the diet.

- The second aim of this part of the study was to investigate the effect of the lower phytate and higher zinc in the organically grown grain on iron and calcium metabolism in the rat, including bone composition.

There was no significant effect of lower phytate and higher zinc on iron metabolism. The absence of antagonism is probably due to the fact that both the iron and the zinc consumed by the rats were present within the diets and not supplemented between meals.

There was also no significant effect of lower endogenous phytate on calcium metabolism. However, the higher endogenous zinc in the organically grown grain resulted in higher fractional apparent absorption of calcium. This was possibly due to the absence of the up-regulation of zinc absorption (present in the rats fed the CONV diet) and the competition between zinc and calcium for absorption sites. The only differences in bone composition seemed to be due to the addition of synthetic minerals which, possibly because they were not already complexed within the grain, interacted with calcium metabolism, particularly with respect to bone composition.

Differences between the diets based on organically and conventionally grown grain that were not explained by the zinc and phytate differences included breath hydrogen production, faecal water content, changes in urinary zinc excretion over time and plasma iron concentration. These indicate that there were other differences between the organically and conventionally grown grain used in the diets that were not elucidated by this study. These differences may involve the carbohydrate fractions of the grain but these have not been investigated in the current study.

Chapter 6

General Discussion

It is important to compare organically and conventionally grown grain in Australia due to the differences in organic farming systems between Australia and Europe, where the concept originated. Factors such as the dry conditions and nutrient depleted soils in Australia have resulted in organic farming systems that are more extensive and with fewer or different inputs in many cases than those in Europe. The comparison of organically and conventionally grown grain in the current study is arguably the most comprehensive study of its kind done in Australia, and, to my knowledge, the world, and is certainly the most extensive due to the number of farms, and hence management regimes, included in the comparison. This study is also unique due to the level of nutritional analysis, including an assessment of grain utilisation by animals.

In this chapter, the main conclusions of this study are presented and discussed in the context of organic farming systems and the consumers of organically and conventionally grown food.

6.1 Plant Nutrition in Organic Systems

The main emerging issue associated with the nutrition of the plants grown within organic systems was that although most nutrients (nitrogen was not assessed) were sufficiently supplied within South Australian organic systems, the addition of rock phosphate fertiliser seemed to be important in order to maintain the phosphorus status of organically grown plants. This amendment was notably absent from organic systems operating in the low rainfall areas of South Australia due to the advice to farmers that the phosphorus present in rock phosphate is not solubilised in the alkaline calcareous soils of these regions. However, although the organic systems within the high rainfall area of this study also had alkaline soils, the application of rock phosphate within these systems seemed to result in sufficient phosphorus levels in the plants.

The fact that the organically managed soils in the high rainfall area were assessed to be depleted of available phosphorus but the plants were phosphorus sufficient indicates that the criteria for phosphorus deficiency in soils may not be appropriate for soils that are organically managed and management-specific criteria may need to be established.

It is possible that even though extractable inorganic phosphorus is low in these farming systems, that the organic phosphorus pool is rapidly turning over and meeting the phosphorus requirements of the plants. Lewis *et al.* (1987) have shown that organic phosphorus accumulates in the top 30 cm of soil at a rate of 2.4-3.5 kg/ha/annum in south eastern South Australia. Lengnick and King (1986) showed (in North America) that organic systems had significantly greater soil organic phosphorus concentration than conventional systems. It may be necessary, therefore, to assess phosphorus status of organically managed soils using both extractable phosphorus and organic phosphorus measurements.

Research is needed, both to determine ways in which soil organic phosphorus pool can be increased and to determine the effectiveness of rock phosphate in enhancing the plant available phosphorus in alkaline calcareous soils, and possibly to identify alternative sources of phosphorus suitable for organic farming systems in these soil types. Without the use of a suitable phosphorus source, the sustainability of organic farming in the low rainfall areas of South Australia can be questioned because phosphorus levels in both the soil and plant tissue from these areas were indicative of phosphorus deficiency. However, if the soil organic phosphorus pool can be maintained or increased, organic systems may be highly sustainable without a suitable inorganic phosphorus source.

Education of organic farmers may also be required because the application of rock phosphate within organic systems may not be directly beneficial in terms of immediate grain yield (Lengnick and King, 1986) but seems to be important in the long term maintenance of the system. However, because organic farmers aim to supply plant nutrients from the soil in a natural manner (and hence focus on the maintenance of soil health) rather than through continuous inputs of soluble fertilisers (NASAA, 1993), organic farmers would most likely be more accepting of the application of rock phosphate without immediate economic benefit than would conventional farmers.

In addition to phosphorus, it is likely that zinc is limiting in some organic systems in South Australia, particularly in the low rainfall areas. However, at least as many conventional systems appeared to be limited by zinc, revealing that zinc deficiency, assessed through plant tissue analysis, is not a result of the system but rather the nutrient deficient nature of soils in South Australia.

Even though zinc as a limiting nutrient is not restricted to organic farming systems, raised awareness amongst organic farmers of the possibility of zinc deficiency may be required. It is important to note that zinc is not uniformly limiting within organic farming systems and therefore the use of plant analysis as a diagnostic tool should be promoted amongst farmers, organic and conventional alike. Foliar application of zinc is permitted under the standards for agricultural production (NASAA, 1993). However, it is not a recommended input and would generally be allowed only for short-term use. Alternative sources of zinc could include crushed rock of various types (NASAA, 1993), although further work may be required to find rock types with suitable zinc concentrations for alleviating, or reducing the effect of, zinc deficiency.

6.2 Nutritional Value of Organically and Conventionally Grown Grain

Two main conclusions were reached in the comparison of the nutritional value of the grain of organically and conventionally grown wheat:

- The organically grown grain generally had lower concentrations of phosphorus (reflected in lower concentrations of phytate), magnesium, potassium and manganese and higher concentrations of zinc and copper compared with conventionally grown grain.
- The combined effect of the higher zinc and lower phytate concentrations in the organically grown grain tested in the feeding experiment, were sufficient to reduce the degree of marginal zinc deficiency possibly induced in weanling rats fed the conventionally grown grain (lower zinc and higher phytate concentrations).

It is an important finding that the differences in zinc and phytate concentrations in organically and conventionally grown grain can result in poorer zinc nutrition for the consumers of conventionally grown grain. The weanling rats in the current study had access to laboratory chow, which is balanced for zinc, while they were still suckling so it is likely that they were not zinc deficient when the treatment diets were applied. In breastfed human infants, a decline in growth can occur due to zinc deficiency at the time when zinc concentrations in breast milk decline (Walravens *et al.*, 1992). If human infants are weaned onto a diet of similar composition to the diet based on conventionally grown grain, marginal zinc deficiency is possible and, if zinc status is already marginal at the time of weaning, more severe zinc deficiency is possible in the long term due to the high zinc requirement during periods of rapid growth. This possibility is supported by evidence of zinc deficiency amongst infants and children, in both developing (Sazawal *et al.*, 1996; Sazawal *et al*, 1998; Umeta *et al.*, 2000; Gibson, 2000) and developed (Walravens *et al.*, 1992; Saha *et al.*, 2000) countries. Alternatively, weaning of infants to a diet of similar composition to the diet based on organically grown grain may be sufficiently higher in bioavailable zinc as to minimise the risk of zinc deficiency.

Apart from children, there are other population groups that are particularly susceptible to zinc deficiency. These include pregnant or lactating women, who have an increased zinc requirement, and vegetarian consumers (Lowik *et al.*, 1990; Kadrabova *et al.*, 1995). For population groups such as these, a diet based on conventionally grown grains similar to that used in the current study could induce, or increase the severity of, zinc deficiency, if it is not supplemented or fortified with zinc.

There are many implications of zinc deficiency, depending on severity. These include compromised immune response (Sazawal *et al.*, 1998; Fraker *et al.*, 2000; Prasad *et al.*, 2000; Saha *et al.*, 2000; Umeta *et al.*, 2000), gastrointestinal disorders (Sazawal *et al.*, 1996), stunted growth (Walravens *et al.*, 1992; Umeta *et al.*, 2000) and foetal abnormalities (Record *et al.*, 1986; King, 2000). It is also possible, as occurs for iron (Zhu and Haas, 1998), that marginal zinc status may result in sub-optimal energy metabolism, and hence energetic inefficiency, due to the number of enzyme systems in which zinc is involved.

Although diets of people in developing countries may approach the high level of cereal consumption simulated by the experimental diets in this study, most populations consume mixed diets, particularly in developed countries. Most mixed diets include some animal products. Meat products are high in available zinc due to the promotion of zinc absorption by an unidentified 'meat factor' (Welch and House, 1995). Vegetarians and the populations of most developing countries, however, obtain the majority of their zinc from cereal grains which, rather than enhancing the absorption of zinc, have limited bioavailability due to the presence of phytate in the grains. Zinc intakes (Ball and Ackland, 2000) and zinc status (Lowik *et al.*, 1990; Kadrabova *et al.*, 1995) of vegetarians, therefore, are in danger of being more marginal than those of omnivores, although not always the case (Ball and Ackland, 2000; Thane and Bates, 2000). Because of the danger of marginal zinc status, it is important for vegetarians to carefully consider their diet in terms of zinc and phytate contents (Oberleas and Harland, 1981). If the present findings are shown to be true generally, vegetarians (and also others at risk of marginal zinc status) could benefit if they choose to consume organically grown wheat, as opposed to conventionally grown wheat. However, further studies are required to confirm the applicability of these findings for humans, before dietary recommendations are made.

The wheat used in the diets of the current study was unrefined, whole grain wheat. Generally, wheat flour that is available for human consumption has been refined to some degree. The highly refined white flours have the hull and aleurone removed. However, even flours marketed as wholemeal have a proportion of these parts of the grain removed. The aleurone contains the majority of both the zinc and phytate present in the wheat grain (O'Dell *et al.*, 1972) but there is some zinc present in the endosperm of the grain and very little phytate (O'Dell *et al.*, 1972). This suggests that refined organically grown wheat, would have lost a good proportion of its zinc and almost all of its phytate compared with whole grain wheat, as would refined conventionally grown wheat. For this reason, a diet similar to the dietary treatment based on organically grown grain in the current study, but using refined grain, may not be capable of preventing marginal zinc deficiency. In the same way, a diet based on refined conventionally grown grain may induce zinc deficiency of greater severity, having also lost zinc in the refining process. However, further research is required to determine whether phytate and zinc are proportionately allocated in a similar way between the grain parts within organically and conventionally grown wheat.

While refinement and processing can alter the content of zinc and phytate and hence the zinc bioavailability of foods, cooking and preparation prior to consumption can also alter bioavailability. Bioavailability of phosphorus from chickpeas has been shown to increase through the simple preparation techniques of soaking and cooking the chickpeas (Nestares *et al.*, 1999), probably due to the activation of endogenous phytase within the chickpeas. It remains to be seen whether baking and soaking affect the bioavailability of zinc from organically and conventionally grown wheat and wheat flour products and whether grain from these contrasting farming systems is similarly affected by cooking and baking techniques.

This study has focused on the negative actions of phytate as an 'anti-nutrient'. However, phytate has also been shown to be of importance in preventing (Thompson and Zhang, 1991) and slowing the development (Jariwalla *et al.*, 1988) of cancer. Also, phosphorus is an essential nutrient and lower levels of non-phytate phosphorus in the organically grown grain may also have biological effects, particularly in growth cell metabolism, due to the importance of phosphorus in DNA and ATP. The implications of these aspects of the lower phosphorus content of organically grown wheat should be investigated in the future.

It is important to remember that the feeding trial in this study did not compare organically and conventionally grown wheat in their entirety, due to the fact that all other nutrients were balanced synthetically, but was designed to compare the zinc and phytate concentrations of these grains. In consideration of nutrient bioavailability and the complexity of nutrient interactions, depending in many cases on the form of nutrient, the synthetic balancing of nutrients other than zinc and phytate may have limited this study. However, due to the length of the feeding trial and the desire to focus on the symptomatology of zinc deficiency only, a diet solely of grain had to be balanced in some way to alleviate other deficiencies. Future research would, almost inevitably, involve the direct comparison of organically and conventionally grown food, without synthetic balancing of nutrients. In such an experiment, the difficulties of the requirement of a nutritionally balanced diet may be overcome by using, for example, organically and conventionally grown soybeans to meet the requirement for the sulphur containing amino acids. However, before such research is undertaken, extensive comparisons of the nutritional profile of organically and conventionally grown soybeans, and other foods used, would be necessary.

Many small (that is, limited in the number of organic systems used), and often uncontrolled (for example, food purchased from supermarket shelves), comparisons of the nutritional composition of organically and conventionally grown food have been done in the past (Pommer and Lepschy, 1985; Leclerc *et al.*, 1991; O'Reilly, 1993; Smith, 1993; Derrick, 1996; Warman and Havard, 1997). However, it is necessary to make extensive comparisons as in the current study, encompassing many of the varied management techniques practised in the two systems. In this way, the true effects of the systems themselves can be distinguished from confounding effects of environment and specific management techniques.

Some of the variables investigated within the feeding trial (for example, breath hydrogen concentration, faecal output and changes in the urinary excretion of zinc), exhibited differences between the diets based on organically and conventionally grown grain that could not be explained by the zinc and phytate contents. This infers that there are other compositional factors, for example carbohydrate and fibre types, which were not investigated in this study but which are influencing the nutritional value of the grain. Certainly, it has been shown that calcium absorption can be enhanced when oligosaccharides are included in the diet (Morohashi *et al.*, 1998; Van den Heuvel *et al.*, 2000). Further comparisons could include these factors.

This study did not take into account the possible presence of herbicide residues in the conventionally grown grain. It is doubtful whether such residues, if present, would have been consistent between the grain from the various conventional systems due to the different herbicide regimes (including no herbicide in some seasons) of the conventional systems investigated. However, the presence of toxins, whether as pesticide residues or naturally occurring toxins (for example, lectins and protease inhibitors (Culliney *et al.*, 1992)) can influence growth and development and even nutrient absorption of consumers. Future research into the nutritional value of organically and conventionally grown wheat, therefore, should also investigate toxins present in the grain.

6.3 Links Between Nutritional Composition of Grain and Organic Farming Practises

It seems likely that the compositional differences in grain detected in the current study are closely related to the core differences between organic and conventional farming systems: the application of herbicides and the addition of soluble phosphatic fertilisers. The interrelationship between these cultural practices and grain nutrient composition are hypothesised to be thus:

- Soluble phosphate fertiliser elevates the phosphorus, and hence phytate, levels in the grain.
- An increase in phosphorus may result in the remobilisation of potassium and magnesium to the grain to form the storage compound, phytate.
- An increase in phosphorus may inhibit the translocation of zinc from the roots to the shoots, and hence to the grain.
- Soluble phosphate fertiliser reduces VAM infection levels and hence possibly the uptake of zinc and copper by the plants.
- Herbicide application, particularly in areas with soils low in zinc or copper, induces zinc and copper deficiencies.

Most of these interrelationships have been shown to exist in conventional systems and become more pronounced as the rate of soluble phosphorus addition or herbicide application increases (Singh *et al.*, 1986; Osborne *et al.*, 1993; Ryan and Ash, 1999). However, further research is required to determine conclusively the degree of influence that each of these factors has on the compositional differences between organically and conventionally grown wheat. By confirming the effect of herbicide application and the addition of soluble phosphatic fertilisers on nutritional composition of conventionally grown wheat, the comparative composition of organically grown wheat, as detected in this study, would be further validated for organic farming systems in general. This is particularly important for those systems utilising management techniques that have not been included in this study, for example the use of biodynamic principles or the application of phosphorus in the form of farmyard manure. Then, organically grown wheat can be promoted with confidence as nutritionally different to conventionally grown wheat and superior in respect to zinc nutrition.

6.4 Implications for Future Research

There are many areas of future research suggested by a study such as this one, including:

- Determination of soil phosphorus critical values for organic farming systems, perhaps including soil organic phosphorus determination, as indicators of sustainability and required phosphorus inputs.
- Nutritional comparisons between organic and conventional farming systems for food crops other than wheat.
- Further analyses of wheat sourced from organic and conventional systems in order to elucidate, and test the consistency of, the differences altering breath hydrogen production, faecal water content, changes in urinary zinc excretion over time and plasma iron concentration. A suggested approach would be to investigate the fibre and carbohydrate fractions.
- A feeding trial using animals with an induced zinc deficiency to assess the effectiveness of a diet based on organically grown wheat in overcoming zinc deficiency.
- A longer term feeding trial to assess the implications of the reduced zinc nutrition provided by the conventionally grown grain (compared with organically grown grain) on animal fertility and the effect on zinc status of subsequent generations.

Appendix 1

Management Practices within each Comparison of Organic and Conventional Farming Systems

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Table A1.1 Details of management for organic (NASAA certified) and conventional systems within comparison A at Wolseley in 1997.

Comparison A (1997)	Organic	Conventional
GPS Location	36 20 55 S, 140 50 02 E	36 20 53 S, 140 49 53 E
Paddock Use in 1994, 1995, 1996	Vetch (hay), Barley, Fallow	Beans, Wheat, Peas
Pre-Sowing Cultivation	4	5
Fertiliser (pre-sowing)	Composted Mineral Fertiliser (0.18 t/ha)	Gypsum (2.5 t/ha)
Sowing Date	10-June	10-June
Seed Dressing	None	None
Fertiliser (at sowing)	Guano (P) (approx. 0.1 t/ha)	DAP (0.1 t/ha) (N:P:K = 18:20:0)
Foliar Spray	None	None
Pest/Weed Control	None	MCPA LV Ester (0.7 l/ha) Ally (5 g/ha) Lontrel (0.1 l/ha)

Table A1.2 Details of management for organic (BFA certified) and conventional systems within comparison B at Wolseley in 1997.

Comparison B (1997)	Organic	Conventional
GPS Location	36 25 64 S, 141 01 63 E	36 25 47 S, 141 59 94 E
Paddock Use in 1994, 1995, 1996	Barley, Beans, Beans+Vetch (grazed and green manured)	Pasture, Wheat, Fallow
Pre-Sowing Cultivation	2	5
Fertiliser (pre-sowing)	None	None
Sowing Date	9-June	9-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	Pivot Mallee Mix (0.07 t/ha) (N:P:K:S+Zn = 8:19:0:3+2)
Foliar Spray	None	None
Pest/Weed Control	None	None

Table A1.3 Details of management for organic (BFA certified) and conventional systems within comparison C at Wolseley in 1997.

Comparison C (1997)	Organic	Conventional
GPS Location	36 21 68 S, 140 57 52 E	36 21 90 S, 140 57 05 E
Paddock Use in 1994, 1995, 1996	Barley, Pasture, Pasture/Silage	Pasture, Barley, Peas
Pre-Sowing Cultivation	1	1
Fertiliser (pre-sowing)	Ecomin (0.2 t/ha) Reactive Phosphate Rock (0.15 t/ha)	None
Sowing Date	10-June	10-June
Seed Dressing	SC27 (Microbes)	None
Fertiliser (at sowing)	None	DAP (0.07 t/ha) (N:P:K = 18:20:0)
Foliar Spray	Zn/Mn Chelate (2 l/ha)	Zn/Mn Chelate (2 l/ha)
Pest/Weed Control	Harrowing (pre-emergence)	Roundup (0.8 l/ha) Tristar (1.2 l/ha) Barrel (1.4 l/ha) Lontrel (0.1 l/ha)

Table A1.4 Details of management for organic (NASAA certified) and conventional systems within comparison D at Nadda in 1997.

Comparison D (1997)	Organic	Conventional
GPS Location	34 38 27 S, 140 56 37 E	34 38 25 S, 140 56 38 E
Paddock Use in 1994, 1995, 1996	Pasture, Pasture, Pasture/Fallow	Pasture/Fallow, Barley, Pasture/Fallow
Pre-Sowing Cultivation	3	3
Fertiliser (pre-sowing)	Basalt Sand (0.05 t/ha)	None
Sowing Date	15-June	15-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	DAP (0.031 t/ha) (N:P:K = 18:20:0)
Foliar Spray	None	None
Pest/Weed Control	None	Ally (4.3 g/ha)

Table A1.5 Details of management for organic (NASAA certified) and conventional systems within comparison E at Nadda in 1997.

Comparison E (1997)	Organic	Conventional
GPS Location	34 40 29 S, 140 57 30 E	34 40 31 S, 140 57 29 E
Paddock Use in 1994, 1995, 1996	Wheat, Pasture, Pasture/Fallow	N/A*, Pasture, Pasture
Pre-Sowing Cultivation	6	3
Fertiliser (pre-sowing)	Basalt Sand (0.05 t/ha)	None
Sowing Date	15-June	15-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	Superphosphate (0.045 t/ha) (N:P:K = 0:17:0)
Foliar Spray	None	None
Pest/Weed Control	None	Amine (500 ml/ha)

*N/A: Information about this year is not available

Table A1.6 Details of management for organic and conventional systems within comparison G at Wudinna in 1997.

Comparison G (1997)	Organic	Conventional
GPS Location	32 50 98 S, 135 09 79 E	32 50 93 S, 135 09 82 E
Paddock Use in 1994, 1995, 1996	Oats, Wheat, Fallow	Wheat, Wheat, Pasture (spray topped)
Pre-Sowing Cultivation	4	0
Fertiliser (pre-sowing)	None	None
Sowing Date	23-June	23-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	DAP+Zn (0.05 t/ha) (N:P:K:S+Zn = 17:19:0:1+5)
Foliar Spray	None	None
Pest/Weed Control	None	None

Table A1.7 Details of management for organic (NASAA certified) and conventional systems within comparison H at Wudinna in 1997.

Comparison H (1997)	Organic	Conventional
GPS Location	33 03 79 S, 135 18 50 E	33 03 80 S, 135 18 46 E
Paddock Use in 1994, 1995, 1996	Wheat, Pasture, Pasture (green manured)	Wheat, Triticale, Pasture
Pre-Sowing Cultivation	2	2
Fertiliser (pre-sowing)	Crushed Mineral Rock (0.042 t/ha)	Zincsol (8 kg/ha)
Sowing Date	23-June	23-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	Superphosphate (0.04 t/ha) (N:P:K = 0:20:0)
Foliar Spray	None	None
Pest/Weed Control	None	Treflan (750 ml/ha) Glean (5 g/ha)

Table A1.8 Details of management for organic (NASAA certified) and conventional systems within comparison I at Wudinna in 1997.

Comparison I (1997)	Organic	Conventional
GPS Location	33 05 04 S, 135 20 11 E	33 05 11 S, 135 20 08 E
Paddock Use in 1994, 1995, 1996	Wheat, Pasture, Pasture	N/A*, N/A, Pasture
Pre-Sowing Cultivation	2	2
Fertiliser (pre-sowing)	Crushed Mineral Rock (0.042 t/ha)	None
Sowing Date	23-June	23-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	Superphosphate (0.045 t/ha) (N:P:K = 0:20:0)
Foliar Spray	None	Zincsol (1.1 kg/ha)
Pest/Weed Control	None	Roundup (250 ml/ha) Treflan (900 ml/ha) Ester 80 (180 ml/ha)

*N/A: Information about this year is not available

Table A1.9 Details of management for organic (NASAA certified) and conventional systems within comparison A at Wolseley in 1998.

Comparison A (1998)	Organic	Conventional
GPS Location	36 20 83 S, 140 50 31 E	36 20 56 S, 140 49 94 E
Paddock Use in 1995, 1996, 1997	Peas (green manured) and Safflower (over summer), Barley, Vetch+Oats (Hay)	Peas, Wheat, Peas
Pre-Sowing Cultivation	5	5
Fertiliser (pre-sowing)	Lime (1.25 t/ha) Soft Rock Phosphate (0.2 t/ha)	DAP (0.07 t/ha) (N:P:K = 18:20:0)
Sowing Date	10-June	10-June
Fertiliser (at sowing)	None	None
Foliar Spray	None	Zincsol (2 l/ha)
Pest/Weed Control	Lely Weeder (8 Aug) (2 passes)	LV Ester 400 (0.5 l/ha) Ally (5 g/ha) Lontrel (0.15 l/ha)

Table A1.10 Details of management for organic (BFA certified) and conventional systems within comparison C at Wolseley in 1998.

Comparison C (1998)	Organic	Conventional
GPS Location	36 22 10 S, 140 57 43 E	36 22 20 S, 140 57 05 E
Paddock Use in 1995, 1996, 1997	Wheat, Pasture, Pasture	Wheat, Peas, Canola
Pre-Sowing Cultivation	3	1
Fertiliser (pre-sowing)	None	None
Sowing Date	10-June	10-June
Fertiliser (at sowing)	Soft Rock Phosphate (0.1 t/ha)	MAP (0.06 t/ha) (N:P:K = 10:22:0)
Foliar Spray	None	None
Pest/Weed Control	Harrowing (pre-emergence)	Pumas Ally (7 g/ha) LV Ester 400 (1 l/ha) Lontrel (0.1 l/ha)

Table A1.11 Details of management for organic (NASAA certified) and conventional systems within comparison D at Nadda in 1998.

Comparison D (1998)	Organic	Conventional
GPS Location	34 39 14 S, 140 56 64 E	34 39 09 S, 140 56 64 E
Paddock Use in 1995, 1996, 1997	Rye (undersown with medic and primrose), Pasture, Pasture/Fallow	Pasture, Barley, Pasture
Pre-Sowing Cultivation	7	3
Fertiliser (pre-sowing)	Basalt Sand (0.06 t/ha)	None
Sowing Date	27-May	27-May
Seed Dressing	None	None
Fertiliser (at sowing)	None	DAP (0.04 t/ha) (N:P:K = 18:20:0)
Foliar Spray	None	None
Pest/Weed Control	None	Ally (4 g/ha) 24D Ester 800 (750 ml/ha)

Table A1.12 Details of management for organic (NASAA certified) and conventional systems within comparison F at Nadda in 1998.

Comparison F (1998)	Organic	Conventional
GPS Location	34 37 92 S, 140 55 41 E	34 37 90 S, 140 55 33 E
Paddock Use in 1995, 1996, 1997	Wheat, Pasture, Pasture/Fallow	N/A*, Pasture, Pasture (spray topped)
Pre-Sowing Cultivation	7	2
Fertiliser (pre-sowing)	Basalt Sand (0.06 t/ha)	None
Sowing Date	27-May	27-May
Seed Dressing	None	None
Fertiliser (at sowing)	None	MAP (0.07 t/ha) (N:P:K = 10:22:0)
Foliar Spray	None	None
Pest/Weed Control	None	Logran (17 g/ha)

*N/A: Information about this year is not available

Table A1.13 Details of management for organic (NASAA certified) and conventional systems within comparison H at Wudinna in 1998.

Comparison H (1998)	Organic	Conventional
GPS Location	33 04 65 S, 135 18 49 E	33 04 61 S, 135 18 48 E
Paddock Use in 1995, 1996, 1997	Wheat, Pasture, Pasture (green manured)	Wheat, Barley, Pasture (spray topped)
Pre-Sowing Cultivation	2	2
Fertiliser (pre-sowing)	Basalt Sand (0.07 t/ha)	Zincsol (7.3 kg/ha)
Sowing Date	10-June	10-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	Superphosphate (0.044 t/ha) (N:P:K = 0:20:0)
Foliar Spray	None	None
Pest/Weed Control	None	Treflan (750 ml/ha) Glean (4.5 g/ha)

Table A1.14 Details of management for organic (NASAA certified) and conventional systems within comparison I at Wudinna in 1998.

Comparison I (1998)	Organic	Conventional
GPS Location	33 03 59 S, 135 20 07 E	33 03 65 S, 135 20 15 E
Paddock Use in 1995, 1996, 1997	Wheat, Pasture, Pasture	N/A*, N/A, Pasture
Pre-Sowing Cultivation	2	1
Fertiliser (pre-sowing)	Basalt Sand (0.07 t/ha)	None
Sowing Date	10-June	10-June
Seed Dressing	None	None
Fertiliser (at sowing)	None	Superphosphate+Zn (0.06 t/ha) (N:P:K+Zn = 0:20:0+2)
Foliar Spray	None	None
Pest/Weed Control	None	None

*N/A: Information about this year is not available

Appendix 2

Soil Test Results for each Comparison of Organic and Conventional Farming Systems

Table A2.1 (a) Results of the soil tests (0-10 cm) comparing organic (Org) and conventional (Conv) systems under high rainfall conditions (Wolseley) in 1997 and 1998.

Year		**1997**			**1998**			
Comparison		**A**	**B**	**C**	**A**	**C**	**Mean**	**F Prob**
pH	**Org**	7.1	7.7	7.6	7.9	7.8	7.6	NS
(CaCl$_2$)	**Conv**	7.7	7.8	7.5	7.7	7.6	7.7	
Organic Carbon								
(%)	**Org**	1.24	1.13	1.13	1.50	1.46	1.29	NS
	Conv	1.23	1.39	1.16	1.48	1.54	1.36	
Colwell P	**Org**	11	18	13	9	20	14.2	P=0.025
(mg/kg)	**Conv**	26	29	14	33	34	27.2	
S	**Org**	14.1	19.2	14.1	6.4	28.0	16.4	NS
(mg/kg)	**Conv**	68.0	9.6	32.7	8.0	16.4	26.9	
Exch. Ca	**Org**	27.2	23.1	27.2	24.2	23.7	25.1	NS
(meq/100g)	**Conv**	26.3	26.5	30.3	27.5	23.4	26.8	
Exch. Mg	**Org**	5.2	5.8	5.8	4.6	5.1	5.3	NS
(meq/100g)	**Conv**	5.7	5.4	6.0	5.5	5.7	5.7	

Table A2.1 (b) Results of the soil tests (0-10 cm) comparing organic (Org) and conventional (Conv) systems under high rainfall conditions (Wolseley) in 1997 and 1998.

Year		1997			1998			
Comparison		**A**	**B**	**C**	**A**	**C**	**Mean**	**F Prob**
Exch. Na	**Org**	0.63	1.00	0.52	0.55	0.53	2.64	NS
(meq/100g)	**Conv**	0.70	0.72	0.57	0.38	0.57	2.38	
Exch. K	**Org**	1.75	1.68	1.95	1.53	1.52	1.69	NS
(meq/100g)	**Conv**	1.55	1.73	1.61	1.68	1.35	1.58	
DTPA Zn	**Org**	0.53	0.66	0.52	0.52	0.57	0.56	NS
(mg/kg)	**Conv**	0.63	1.33	1.06	0.71	0.51	0.85	
DTPA Cu	**Org**	0.58	0.57	0.69	0.82	0.84	0.70	P=0.044
(mg/kg)	**Conv**	0.62	0.42	0.52	0.60	0.70	0.57	
DTPA Mn	**Org**	4.26	3.52	4.36	4.35	4.89	4.28	NS
(mg/kg)	**Conv**	2.94	4.12	5.83	4.62	8.05	5.11	
DTPA Fe	**Org**	12	14	11	14	20	14	NS
(mg/kg)	**Conv**	13	14	15	15	20	15	

Table A2.2 (a) Results of the soil tests (0-10 cm) comparing organic (Org) and conventional (Conv) systems under marginal rainfall conditions (Nadda and Wudinna) in 1997 and 1998.

Year		**1997**					**1998**					
Comparison		**D**	**E**	**G**	**H**	**I**	**D**	**F**	**H**	**I**	**Mean**	**F Prob**
pH (CaCl$_2$)	**Org**	7.8	7.5	7.9	7.8	7.9	5.9	5.7	8.0	7.5	7.3	NS
	Conv	7.8	7.6	7.8	7.7	7.8	5.8	5.7	7.9	6.9	7.2	
Organic Carbon (%)	**Org**	0.85	0.74	1.03	2.34	1.87	0.58	0.42	1.70	0.82	1.15	P=0.016
	Conv	0.88	0.63	0.95	1.84	1.30	0.44	0.45	1.40	0.39	0.92	
Colwell P (mg/kg)	**Org**	15	19	18	23	10	9	10	20	5	14.3	P=0.051
	Conv	16	11	48	22	22	21	25	27	12	22.7	
S (mg/kg)	**Org**	7.9	12.5	24.3	20.4	27.3	3.2	2.3	5.2	2.6	11.7	NS
	Conv	8.0	6.5	23.0	20.5	16.0	3.8	3.1	6.5	4.0	10.2	
Exch. Ca (meq/100g)	**Org**	13.2	8.6	16.8	21.5	19.6	2.8	2.7	18.6	6.1	12.2	P=0.004
	Conv	10.9	6.4	16.3	18.7	18.4	2.3	2.1	13.7	3.4	10.2	
Exch. Mg (meq/100g)	**Org**	1.96	1.89	1.93	2.44	3.55	1.03	1.16	2.34	0.84	1.90	P=0.050
	Conv	1.76	1.50	2.19	2.52	2.51	0.75	0.98	1.66	0.60	1.61	

Table A2.2 (b) Results of the soil tests (0-10 cm) comparing organic (Org) and conventional (Conv) systems under marginal rainfall conditions (Nadda and Wudinna) in 1997 and 1998.

Year		1997					1998					
Comparison		**D**	**E**	**G**	**H**	**I**	**D**	**F**	**H**	**I**	**Mean**	**F Prob**
Exch. Na	**Org**	0.13	0.14	0.39	0.42	0.70	0.06	0.05	0.10	0.02	0.22	NS
(meq/100g)	**Conv**	0.15	0.06	0.31	0.68	0.41	0.06	0.06	0.05	0.15	0.21	
Exch. K	**Org**	1.65	1.38	2.36	1.74	2.17	0.54	0.55	1.57	0.31	1.36	NS
(meq/100g)	**Conv**	1.44	0.93	2.61	1.58	2.18	0.43	0.63	0.46	0.31	1.17	
DTPA Zn	**Org**	0.49	0.91	0.65	1.39	0.95	0.28	0.26	0.49	0.24	0.63	NS
(mg/kg)	**Conv**	0.41	0.39	1.39	1.04	0.99	1.01	0.42	0.89	0.21	0.75	
DTPA Cu	**Org**	0.33	0.19	0.40	0.60	0.71	0.28	0.23	0.81	0.19	0.42	NS
(mg/kg)	**Conv**	0.32	0.16	0.51	0.49	0.97	0.24	0.32	0.50	0.17	0.41	
DTPA Mn	**Org**	12.5	12.8	12.2	8.57	11.7	9.99	9.75	10.29	7.55	10.59	NS
(mg/kg)	**Conv**	14.0	10.0	17.8	8.38	12.1	1.04	12.09	7.69	5.81	9.87	
DTPA Fe	**Org**	3	5	2	6	5	11	13	7	6	7	NS
(mg/kg)	**Conv**	3	4	3	4	5	18	23	5	10	8	

Appendix 3

Detailed Procedures for Tissue and Grain Analysis

A3.1 Elemental Analysis

The elemental analyses were done by Waite Analytical Services, The University of Adelaide, Waite Campus (L. Palmer and T.O. Fowles).

Plant material (0.6 g) was left overnight in 10 ml concentrated nitric acid and then digested (based on Zarcinas *et al.*, 1987) for 25 minutes at 80 •C, 20 minutes at 100 •C, 90 minutes at 125 •C and finished off at 140 •C until 1 ml of solution remained (approximately 170 minutes). For the straw and chaff samples, the digest was slowed to prevent spitting caused by their high silica content. These samples (0.4 g of plant material with 6 ml of nitric acid) were digested in the same way initially but held at 125 •C for 120 minutes and finished off at 130 •C.

Grain samples (0.8 g) were left overnight and then digested in 10 ml of nitric acid and 1 ml of perchloric acid for 20 minutes at each of 80, 100 and 120 •C, then 60 minutes at 130 •C, 30 minutes at 140 •C, 50 minutes at 150 •C, 40 minutes at 170 •C, 15 minutes at 180 •C and finished off for 7 minutes at 225 •C.

The digested samples were cooled and diluted to 25 ml using 1% nitric acid. They were analysed for iron, manganese, copper, zinc, calcium, magnesium, sodium, potassium, phosphorus and sulphur concentrations using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES). The machine used was an ARL 3580 B ICP with the plasma in radial mode and using liquid nebulisation.

A3.2 Phytate Analysis

The phytate analyses were done by the Plant, Soil and Nutrition Laboratory, USDA-ARS, Cornell University Campus (R.M. Welch and L.I. Heller).

To prepare the samples for phytate analysis, 0.25g of ground grain was extracted in 10 ml of 0.5M HCl for two hours (method of Lehrfeld, 1994). Following centrifugation, the supernatant was diluted to a 10% solution using milli-Q water.

Phytate analysis was carried out on a Dionex liquid chromatograph system controlled by PeakNet software (modified from Dionex Application Note 65 (Dionex, 2000)). Three eluents, water (milli-Q), 200 mM sodium hydroxide (carbonate free as described by

Dionex Application Note 65) and 50% methanol, were used with a flow rate of 1 ml/min and the operation program in Table A3.1.

Table A3.1 The operation program for the analysis of phytate (inositol hexaphosphate) using a Dionex liquid chromatograph system.

	Percentage of:		
Time (min)	**Water**	**Sodium Hydroxide**	**Methanol**
0.0	83	12	5
11.0	35	60	5
11.1	83	12	5
15.0	83	12	5

A3.3 Amino Acid Analysis

The amino acid analyses were carried out by the Nucleic Acid and Protein Chemistry Unit, The University of Adelaide, Waite Campus (N. Shirley and J. Lahnstein).

To prepare for amino acid analysis, the samples underwent liquid phase hydrolysis in 6M hydrochloric acid with phenol (to protect the tyrosine) and dithiodipropionic acid (to stabilise the cysteine) for 16 hours at 116 •C (based on Barkholt and Jensen (1989)). Pre-column derivatisation was carried out using o-phthalaldehyde and 9-fluorenylmethyl chloroformate as described by Blankenship *et al.* (1989). Amino acid analysis was carried out on a Hewlett-Packard AminoQuant II amino acid analyser (method described by AminoQuant, 1990). This instrument consists of an HP 1090 Series II liquid chromatograph controlled by HP ChemStation software.

Appendix 4

Concentrations of Iron, Calcium, Sodium and Sulphur in Plant Tissue and Grain Grown within Organic and Conventional Farming Systems

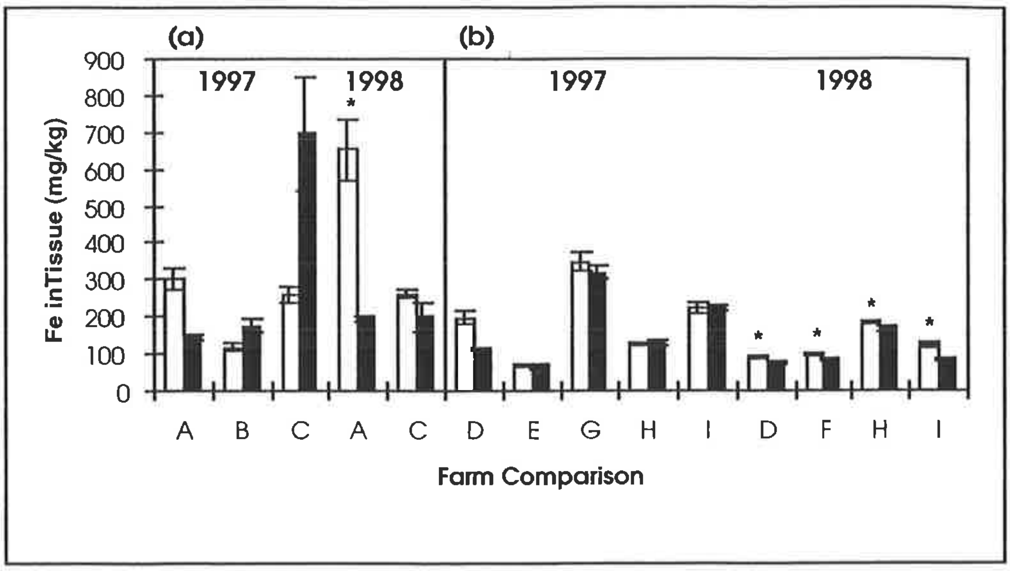


Figure A4.1 The iron concentration at late tillering of the whole tops of plants grown in organic (☐) and conventional (■) farming systems in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$). The high iron in the organic system of comparison A (1998) was due to soil contamination of the wheat shoots from the use of a mechanical weeder prior to sampling. Other values greater than 300 mg/kg may also be due to soil contamination of samples.

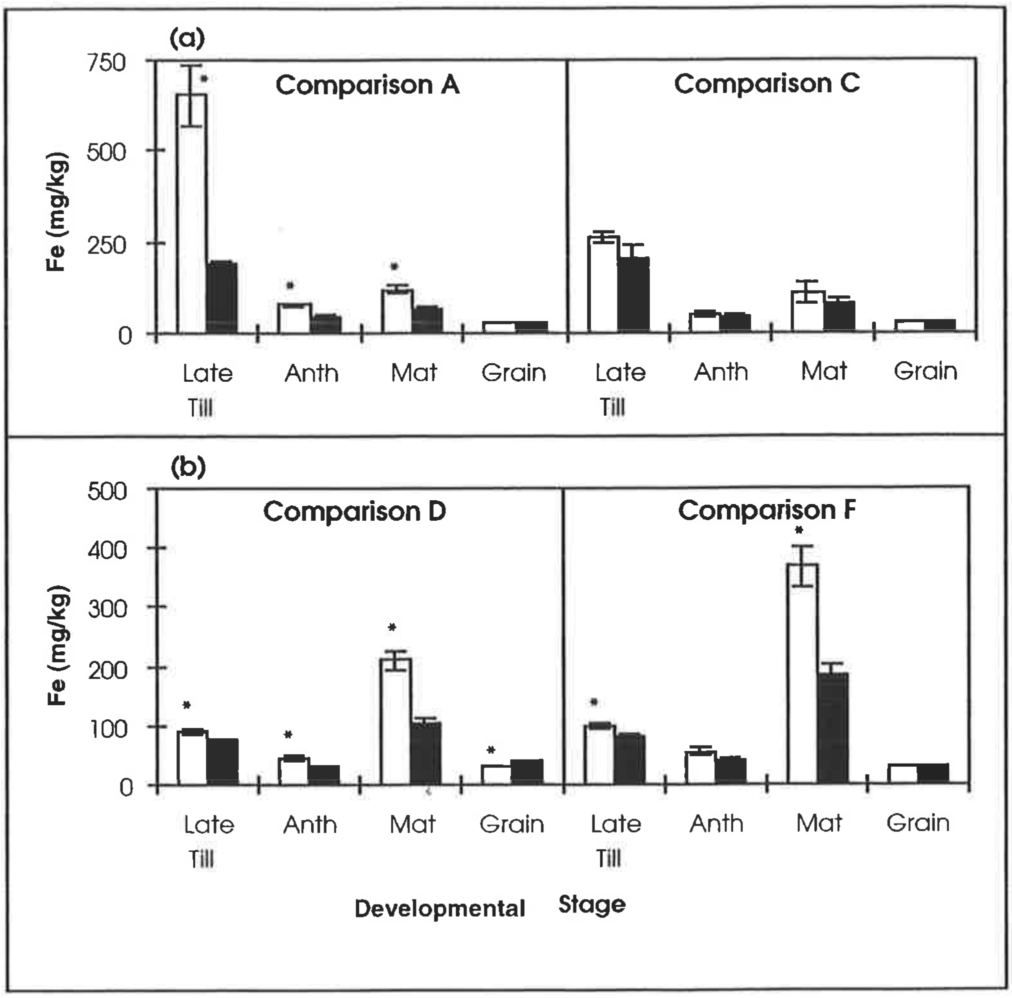


Figure A4.2 The iron concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of plants grown in organic (☐) and conventional (■) farming systems in high (a) and low (b) rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$). The high iron in the organic system of comparison A (1998) was due to soil contamination with the use of a mechanical weeder prior to sampling. Other values greater than 300 mg/kg may also be due to soil contamination of samples.

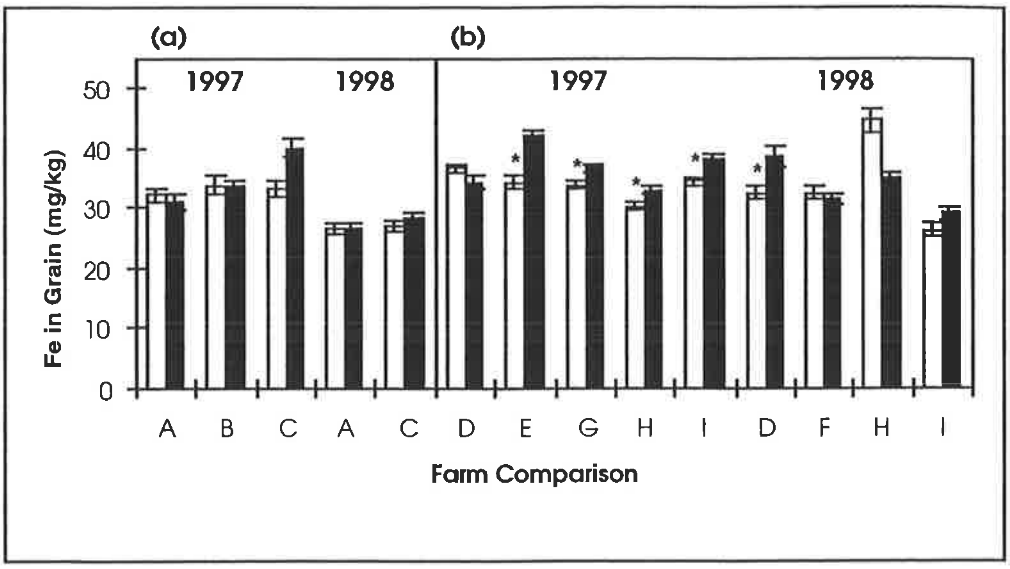


Figure A4.3 The iron concentration in the grain of wheat grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

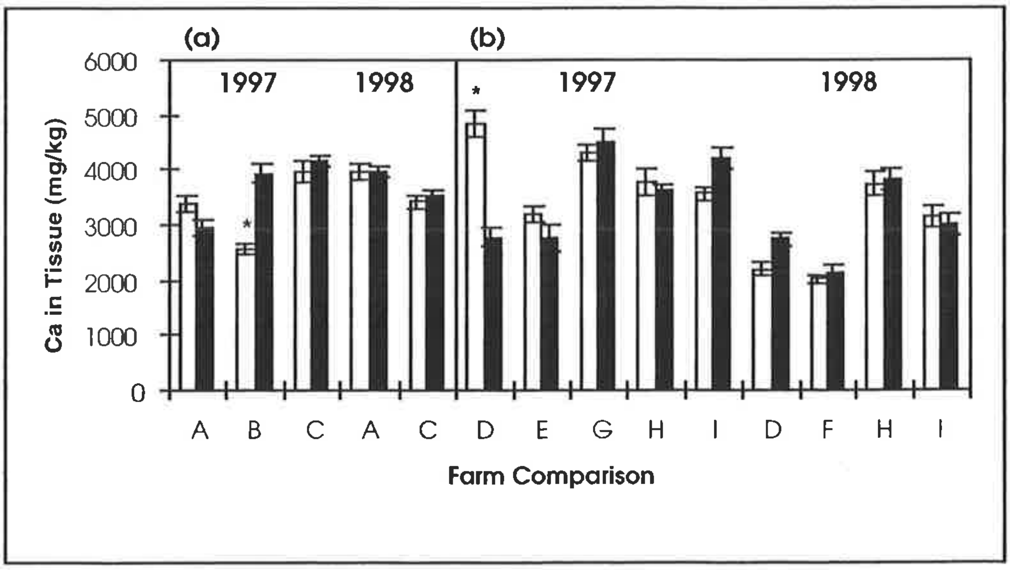


Figure A4.4 The calcium concentration at late tillering of the whole tops of plants grown in organic (□) and conventional (■) farming systems in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

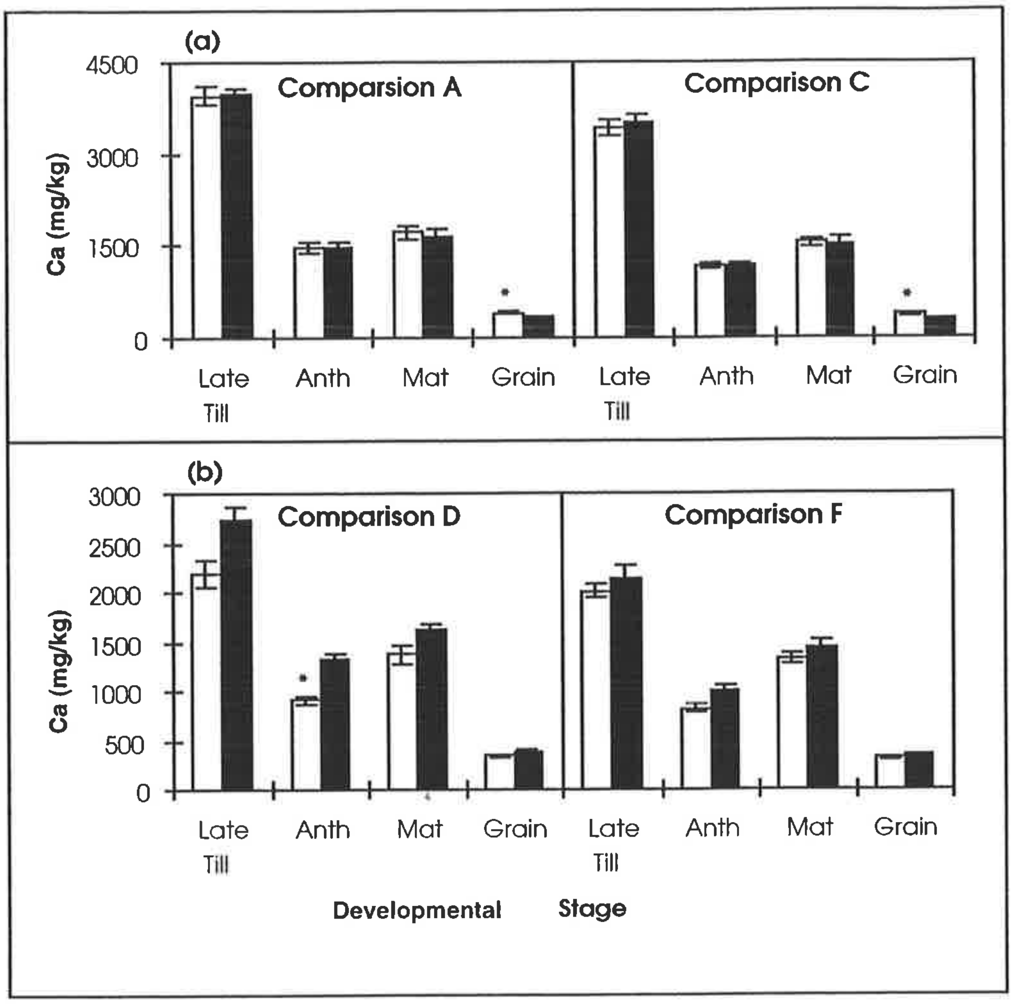


Figure A4.5 The calcium concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of plants grown in organic (□) and conventional (■) farming systems in high (a) and low (b) rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$).

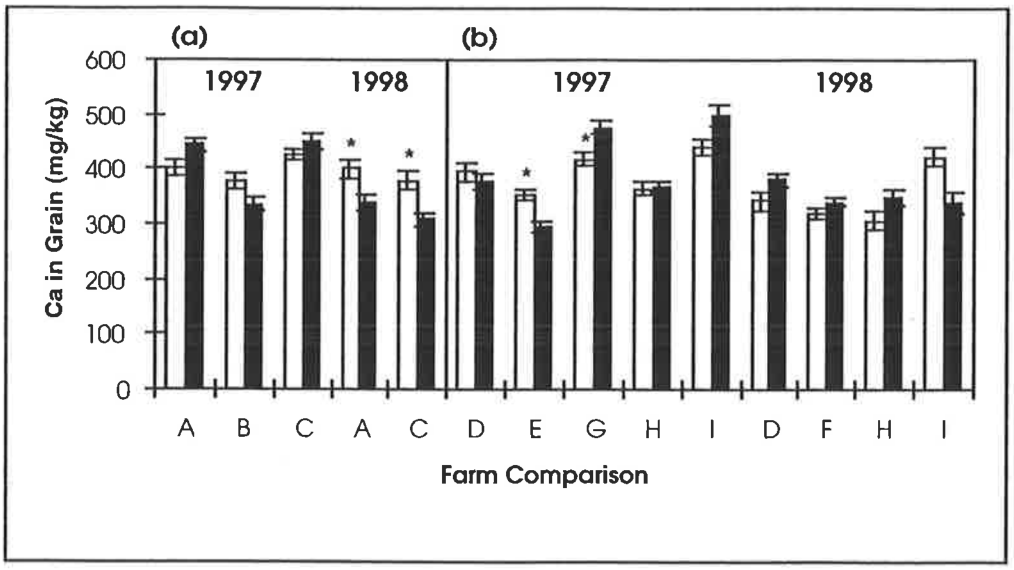


Figure A4.6 The calcium concentration in the grain of wheat grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

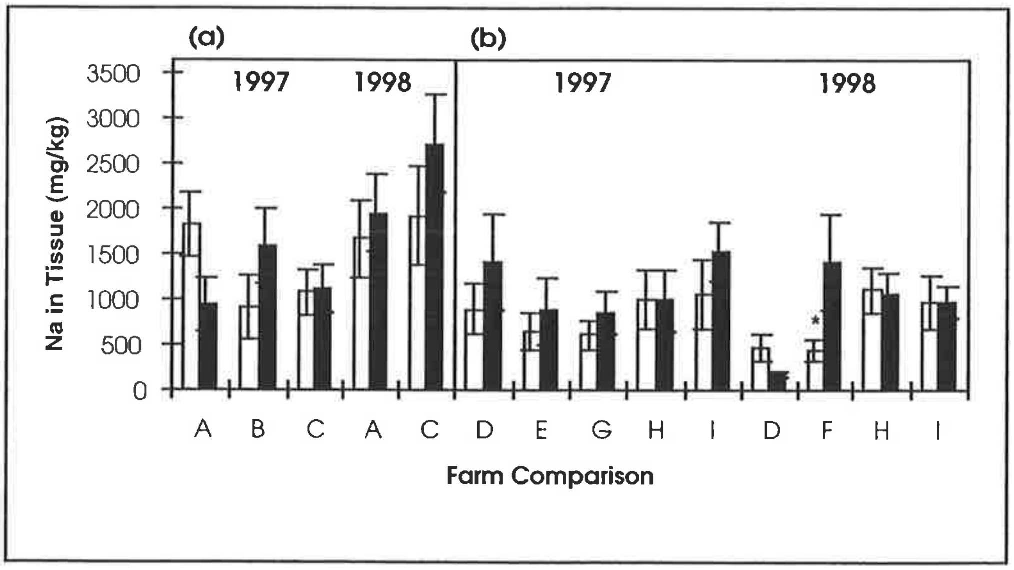


Figure A4.7 The sodium concentration at late tillering of the whole tops of plants grown in organic (□) and conventional (■) farming systems in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

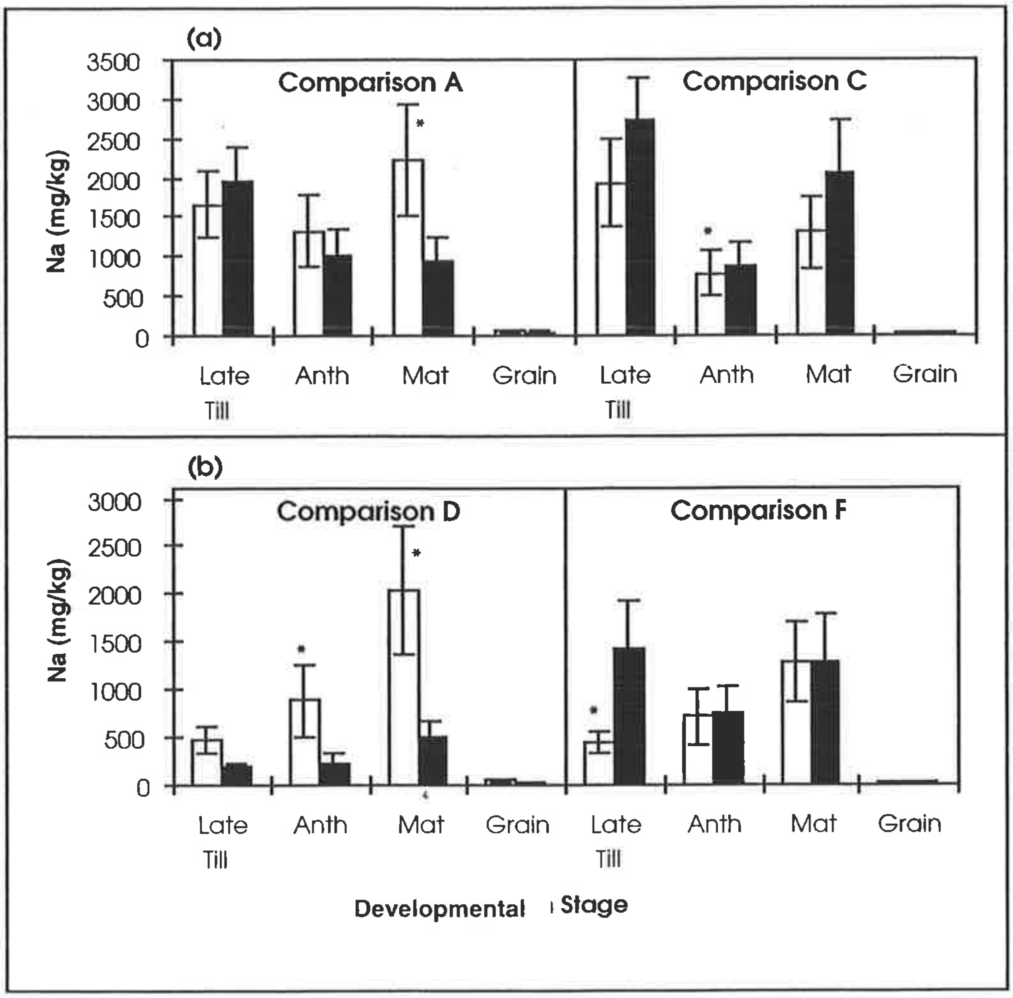


Figure A4.8 The sodium concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of plants grown in organic (☐) and conventional (■) farming systems in high (a) and low (b) rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$).

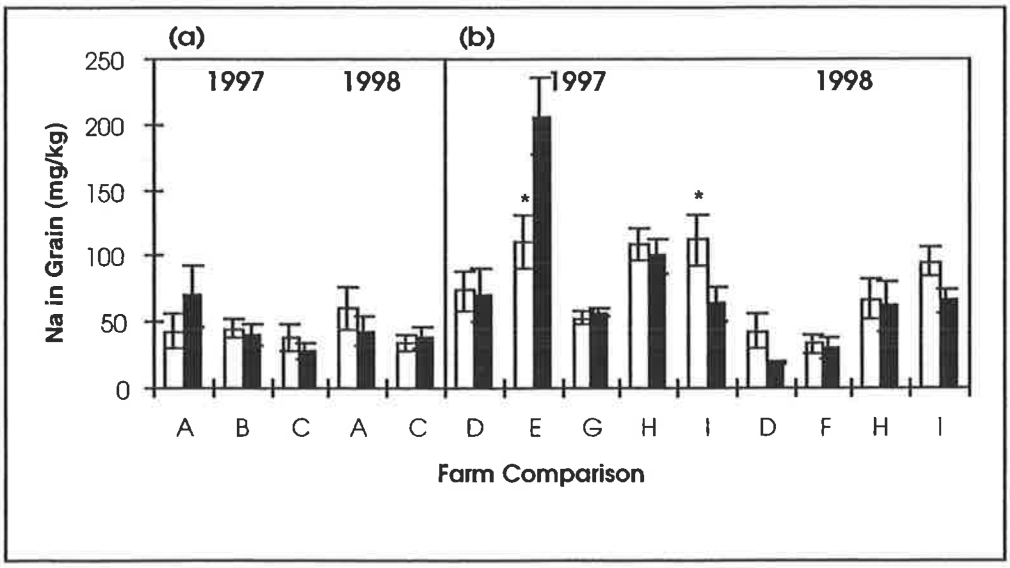


Figure A4.9 The sodium concentration in the grain of wheat grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

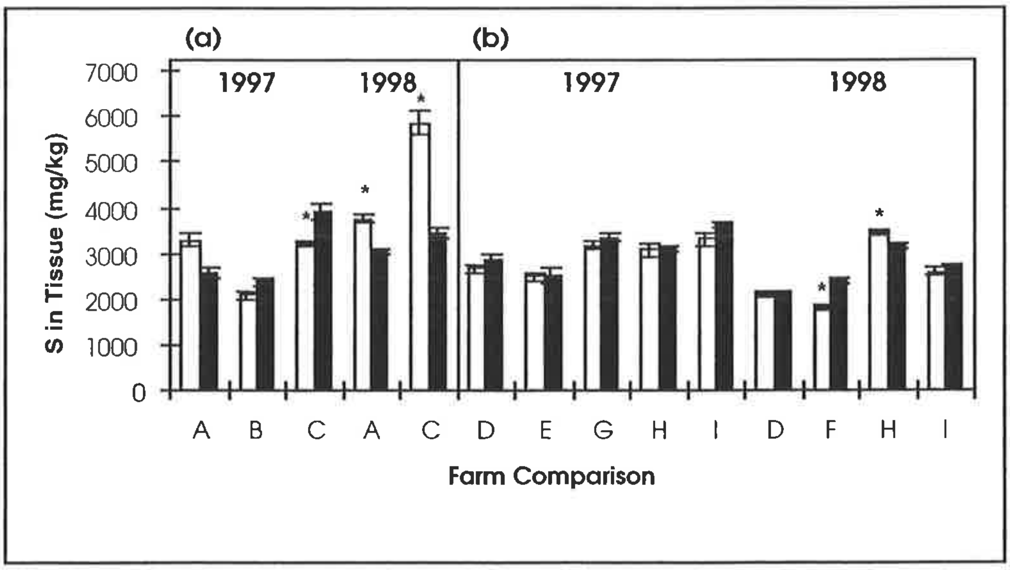


Figure A4.10 The sulphur concentration at late tillering of the whole tops of plants grown in organic (□) and conventional (■) farming systems in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

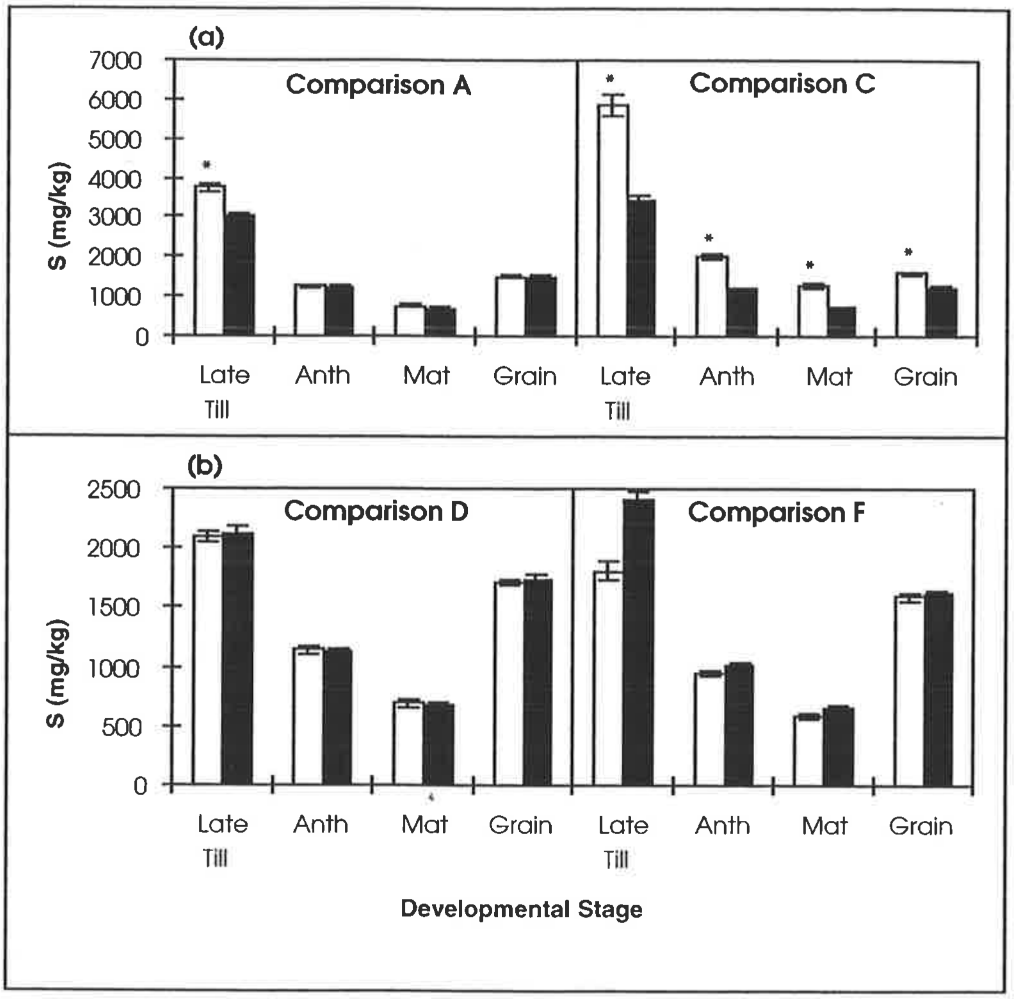


Figure A4.11 The sulphur concentration in the whole tops at late tillering (Late Till) and anthesis (Anth) and in the straw (Mat) and grain at maturity of plants grown in organic (□) and conventional (■) farming systems in high (a) and low (b) rainfall environments in 1998. The asterisk indicates that the organic system was significantly different from the conventional system within a developmental stage ($P<0.05$).

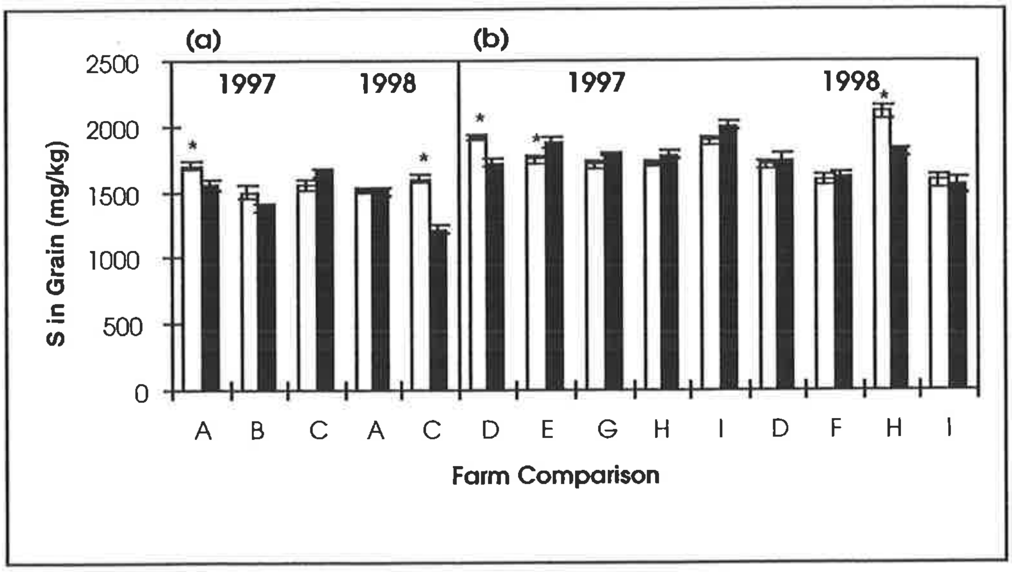


Figure A4.12 The sulphur concentration in the grain of wheat grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

Appendix 5

Total Above Ground Nutrient Uptake per Hectare in Organic and Conventional Farming Systems

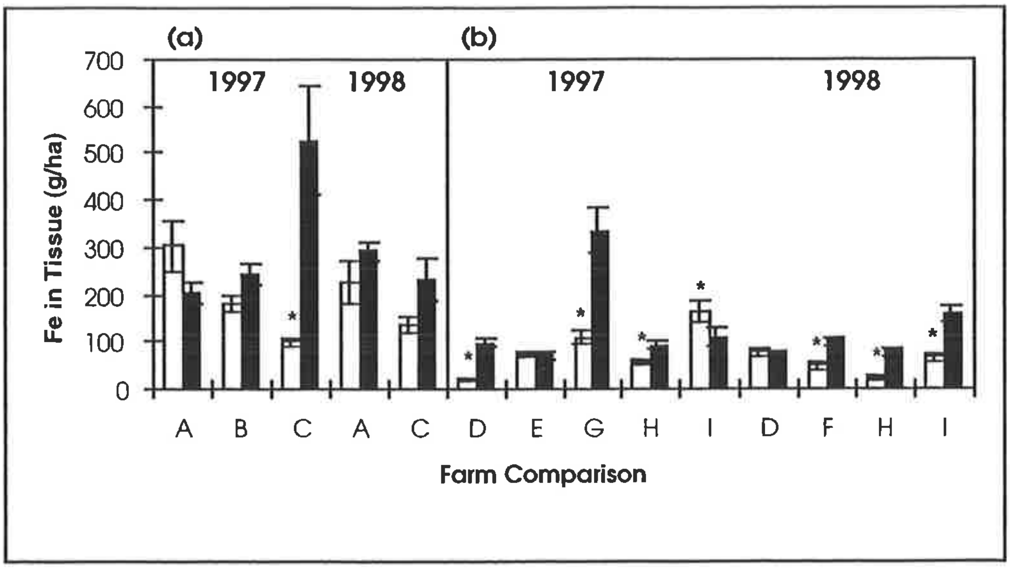


Figure A5.1 The above ground iron uptake per hectare at late tillering of wheat plants grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05). Values greater than 300 mg/kg may be due to soil contamination of samples.

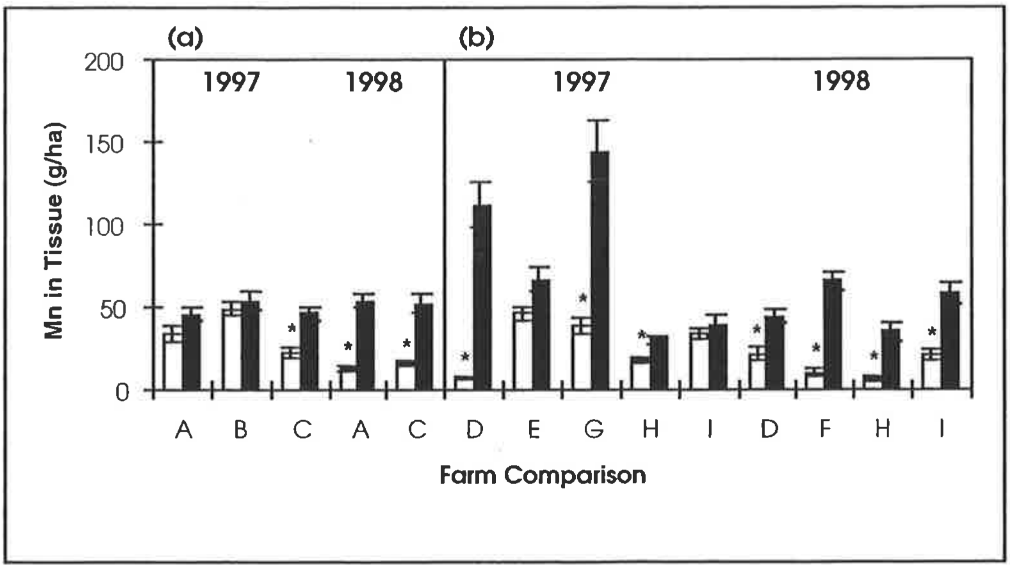


Figure A5.2 The above ground manganese uptake per hectare at late tillering of wheat plants grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

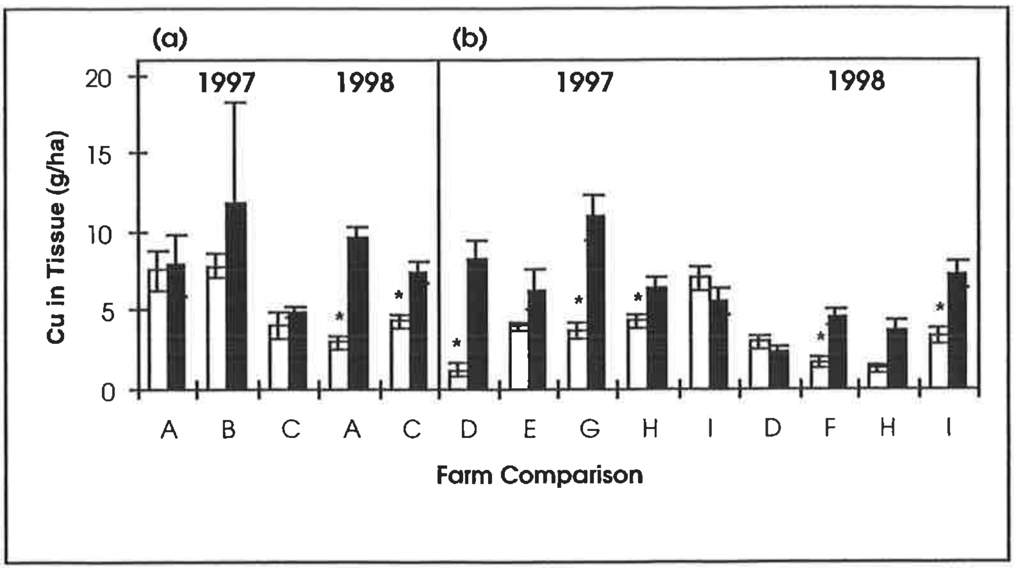


Figure A5.3 The above ground copper uptake per hectare at late tillering of wheat plants grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

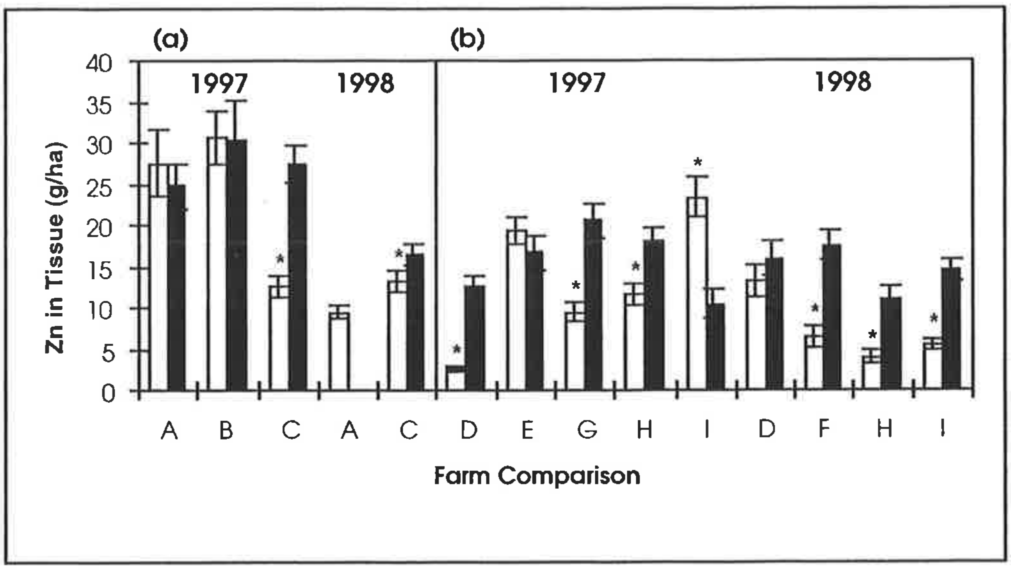


Figure A5.4 The above ground zinc uptake per hectare at late tillering of wheat plants grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$). Data from the conventional system of comparison A (1998) (averaged 164.7 g/ha) has been excluded from the ANOVA due to contamination by foliar zinc application prior to sampling.

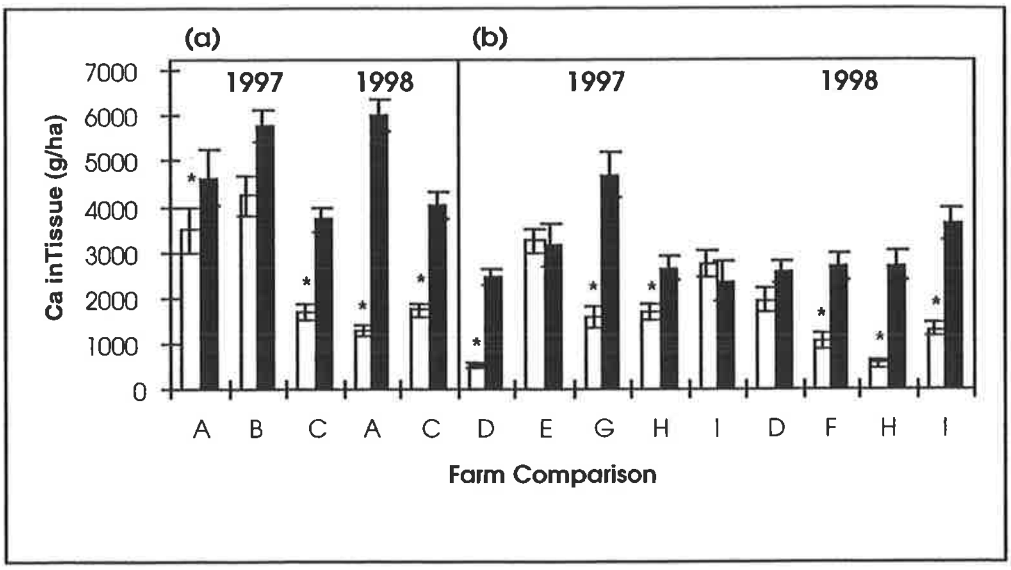


Figure A5.5 The above ground calcium uptake per hectare at late tillering of wheat plants grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

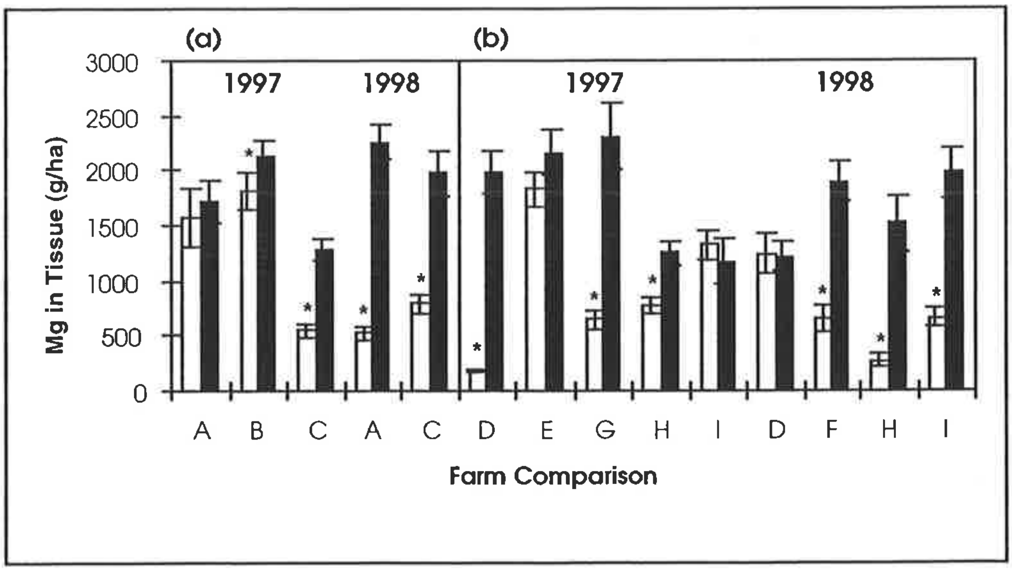


Figure A5.6 The above ground magnesium uptake per hectare at late tillering of wheat plants grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

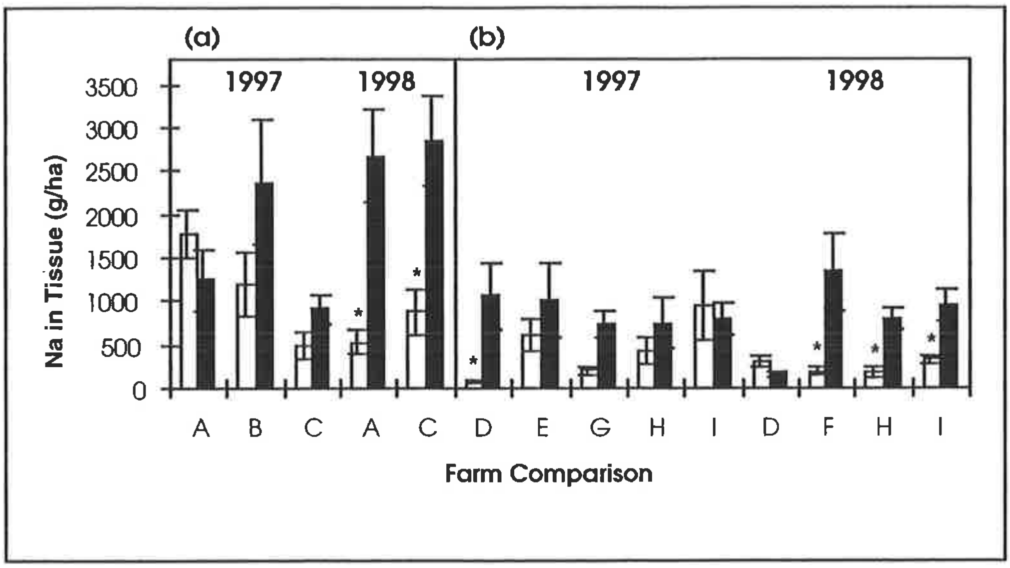


Figure A5.7 The above ground sodium uptake per hectare at late tillering of wheat plants grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

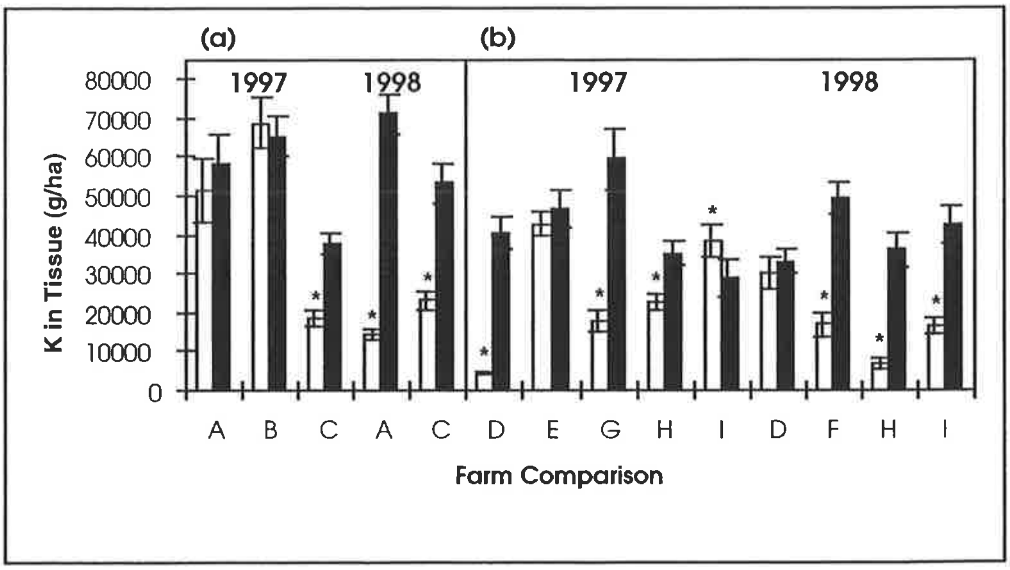


Figure A5.8 The above ground potassium uptake per hectare at late tillering of wheat plants grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

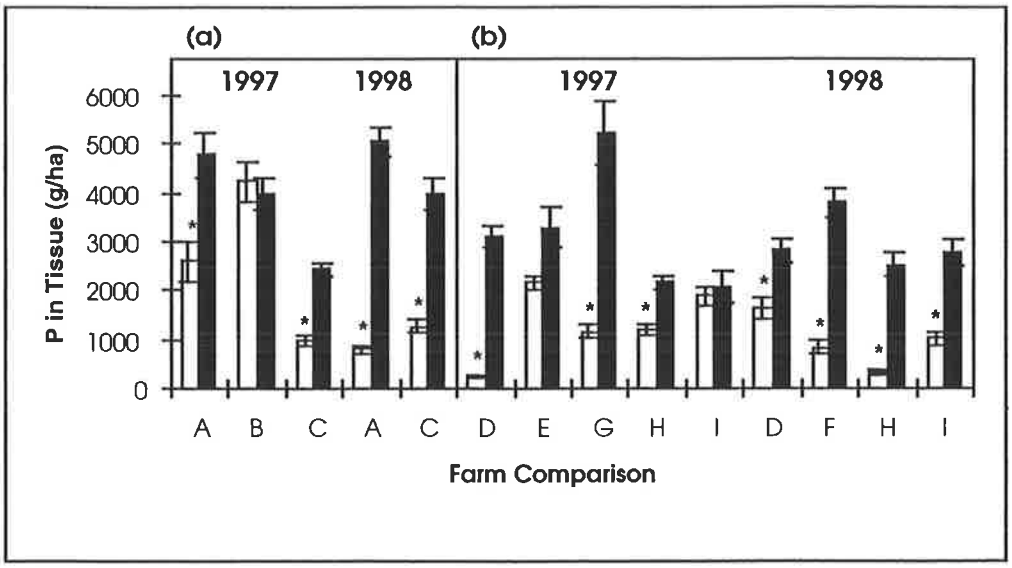


Figure A5.9 The above ground phosphorus uptake per hectare at late tillering of wheat plants grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

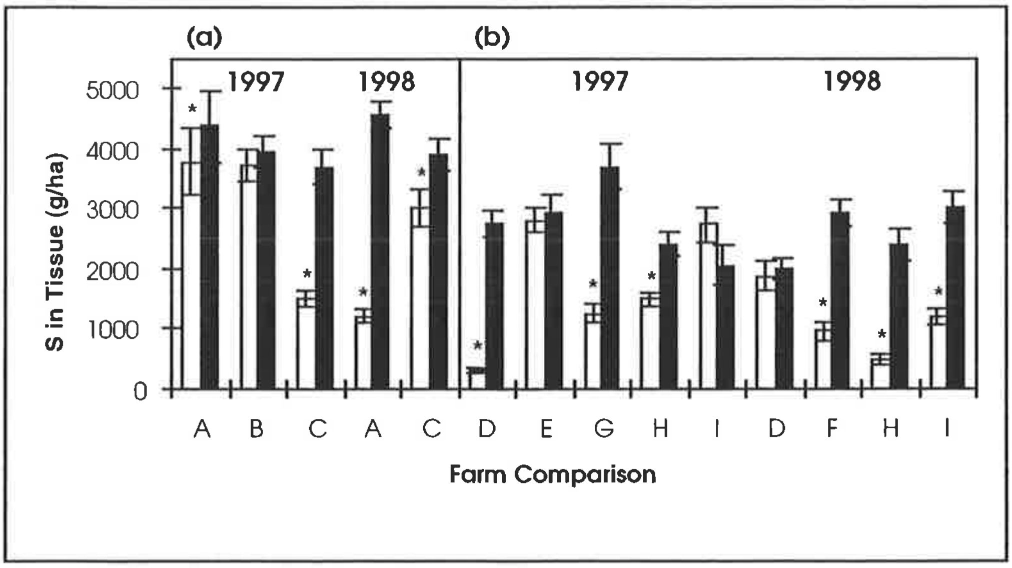


Figure A5.10 The above ground sulphur uptake per hectare at late tillering of wheat plants grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

Appendix 6

Nutrient Removal via Grain from Organic and Conventional Farming Systems

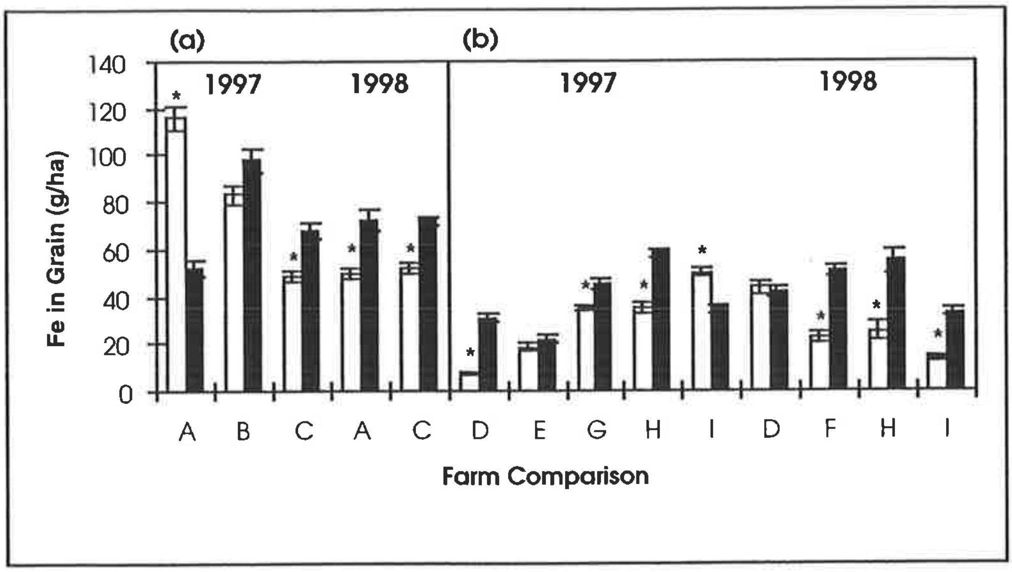


Figure A6.1 The total iron content at maturity in grain organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

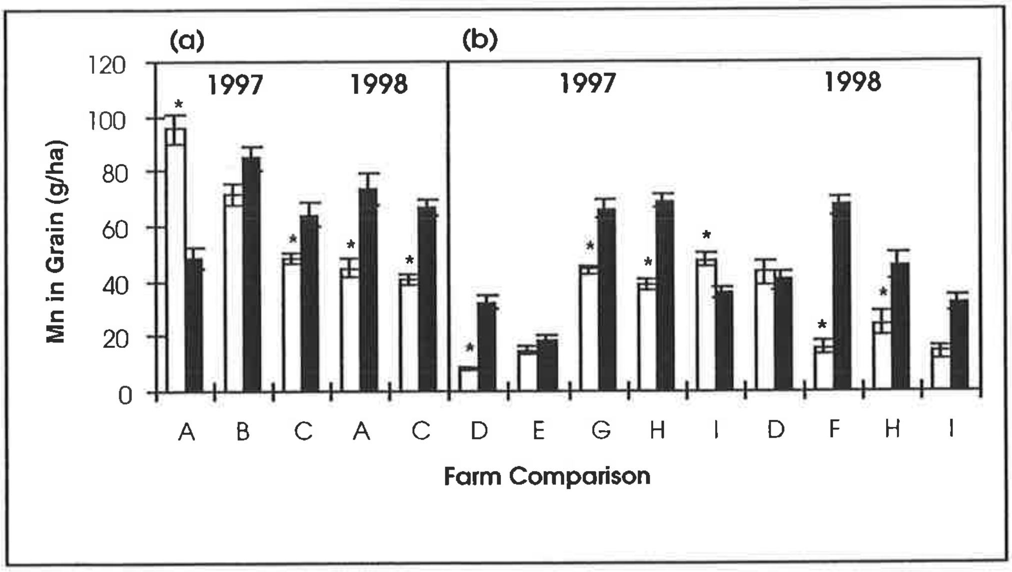


Figure A6.2 The total manganese content at maturity in grain grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

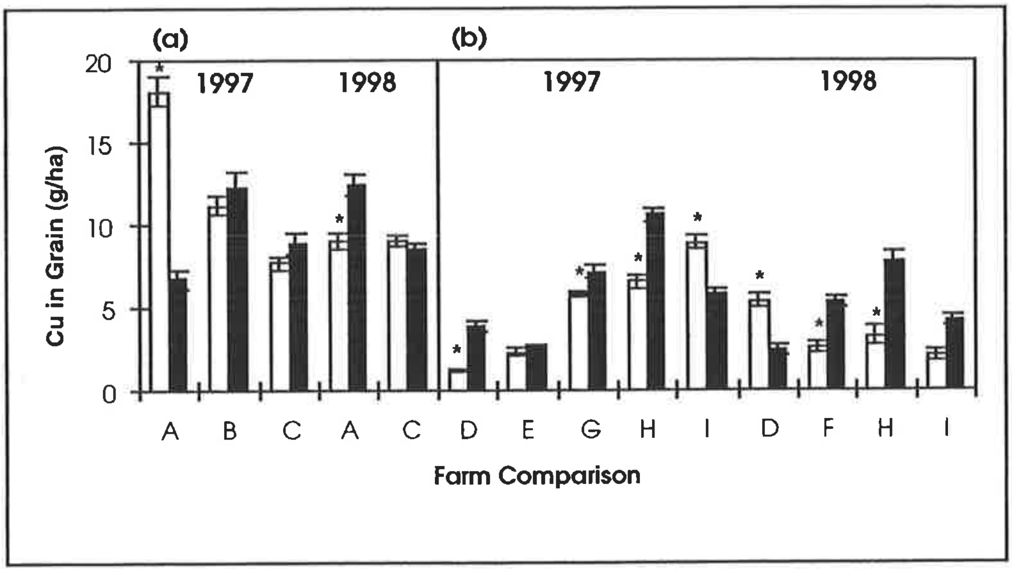


Figure A6.3 The total copper content at maturity in grain grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

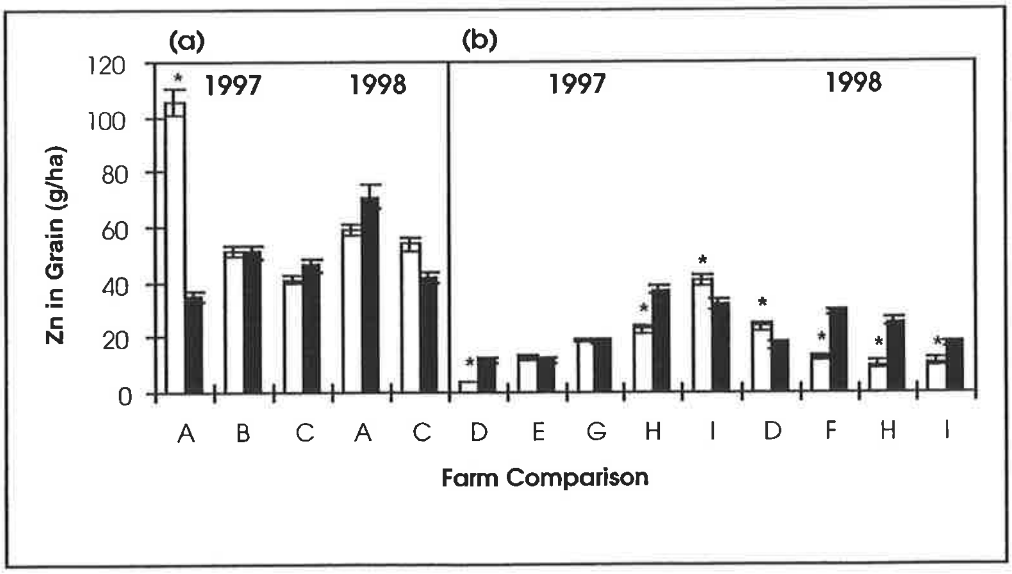


Figure A6.4 The total zinc content at maturity in grain grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison ($P<0.05$).

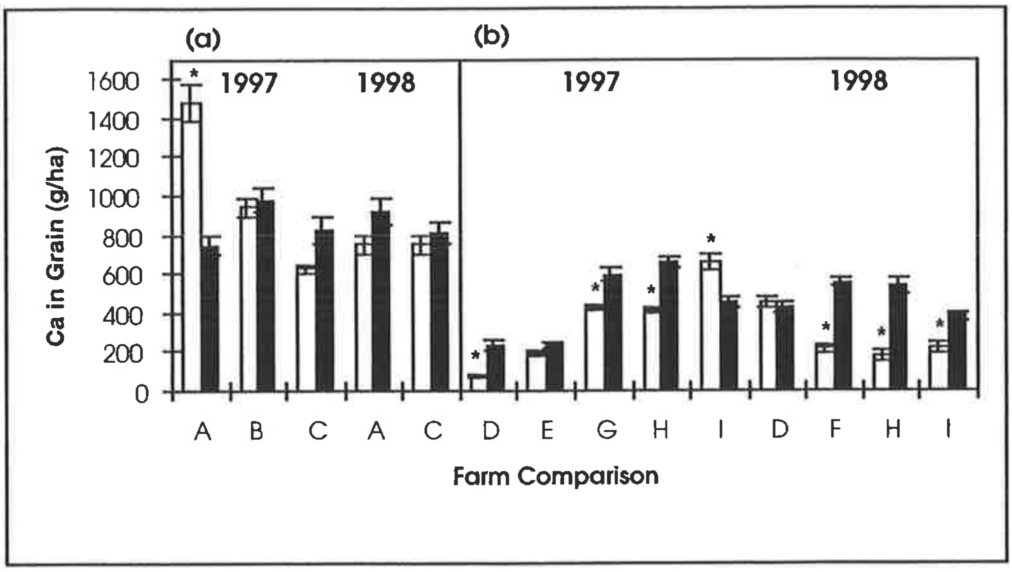


Figure A6.5 The total calcium content at maturity in grain grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

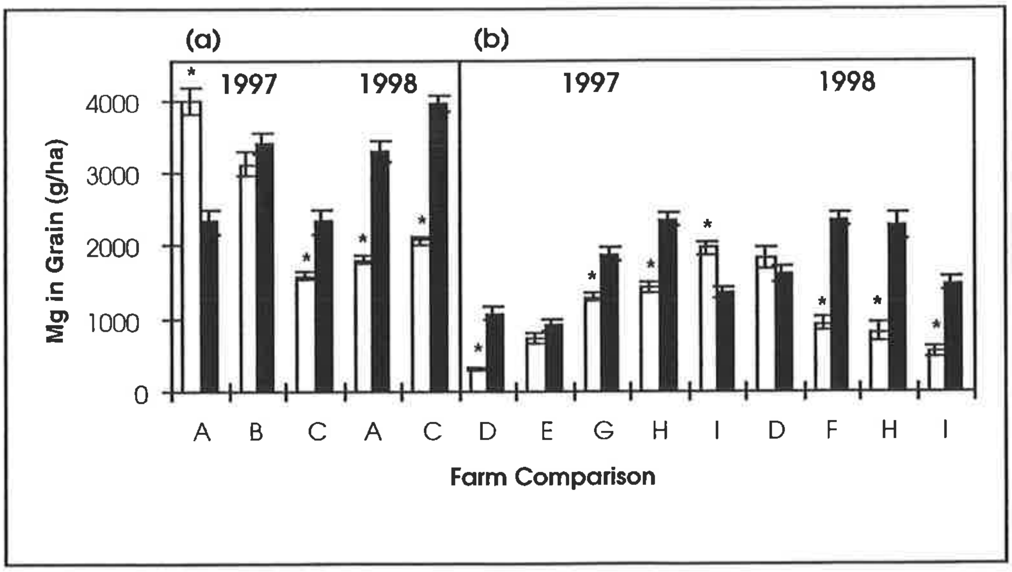


Figure A6.6 The total magnesium content at maturity in grain grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

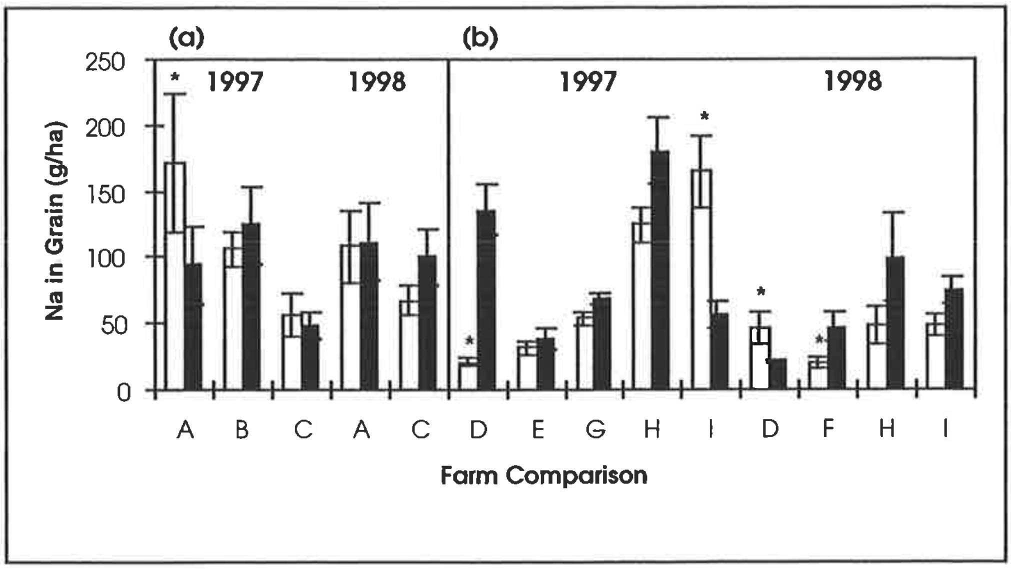


Figure A6.7 The total sodium content at maturity in grain grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

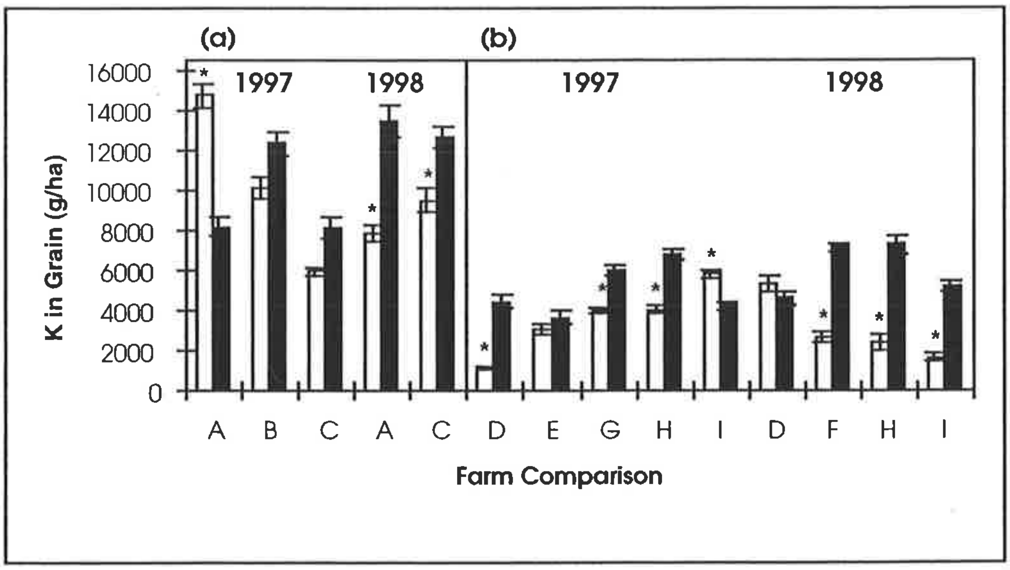


Figure A6.8 The total potassium content at maturity in grain grown organically (□) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

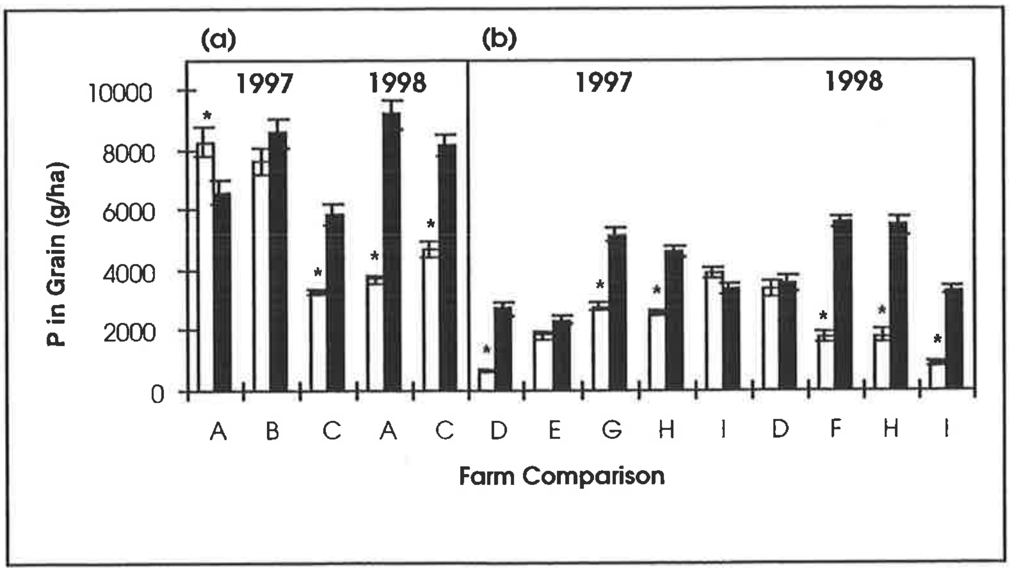


Figure A6.9 The total phosphorus content at maturity in grain grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

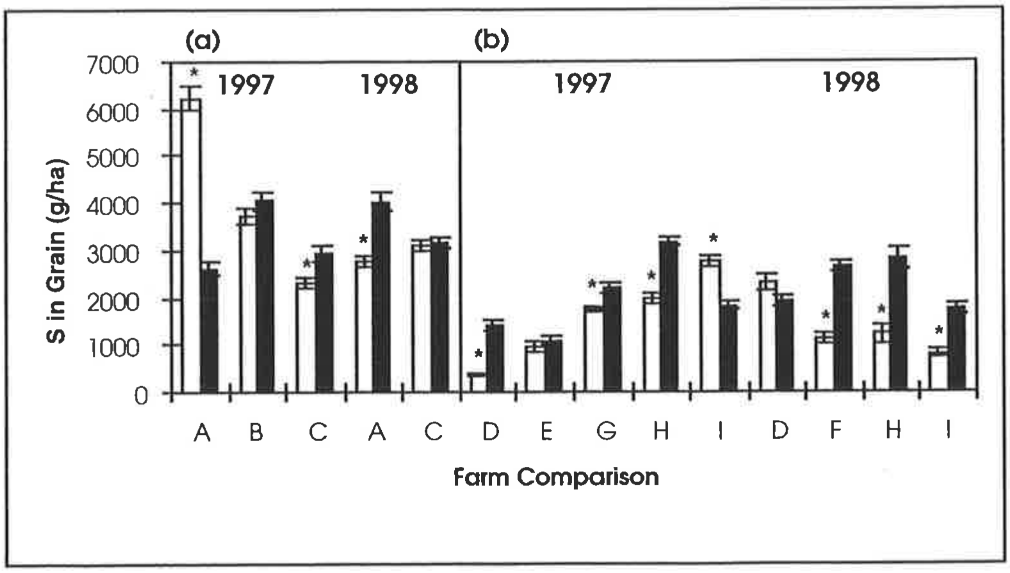


Figure A6.10 The total sulphur content at maturity in grain grown organically (☐) and conventionally (■) in high (a) and low (b) rainfall environments. The asterisk indicates that the organic system was significantly different from the conventional system within a comparison (P<0.05).

Appendix 7

Amino Acid Concentrations in Organically and Conventionally Grown Wheat

Table A7.1 Amino Acid concentrations (g/100 g (DW) as protein amino acids) in grain (varieties Janz and Trident) grown within organic and conventional farming systems in high rainfall comparisons (n=8). Values in parentheses are the standard errors.

Amino Acid	Organic		Conventional	
	Janz	Trident	Janz	Trident
Ala	0.39 (0.02)	0.39 (0.01)	0.37 (0.02)	0.36 (0.01)
Arg	0.62 (0.03)	0.61 (0.02)	0.59 (0.03)	0.55 (0.02)
Asx*	0.59 (0.02)	0.59 (0.01)	0.58 (0.02)	0.55 (0.02)
Cys	0.28 (0.01)	0.28 (0.01)	0.27 (0.01)	0.25 (0.01)
Glx**	3.45 (0.12)	3.51 (0.12)	3.21 (0.16)	2.95 (0.12)
Gly	0.42 (0.01)	0.40 (0.01)	0.40 (0.02)	0.35 (0.01)
His	0.31 (0.01)	0.31 (0.01)	0.28 (0.01)	0.27 (0.01)
Ile	0.46 (0.02)	0.46 (0.01)	0.43 (0.02)	0.41 (0.02)
Leu	0.81 (0.03)	0.80 (0.02)	0.76 (0.03)	0.71 (0.03)
Lys	0.27 (0.01)	0.26 (0.01)	0.26 (0.01)	0.24 (0.01)
Met	0.23 (0.01)	0.24 (0.00)	0.23 (0.01)	0.22 (0.01)
Phe	0.60 (0.02)	0.63 (0.02)	0.56 (0.03)	0.54 (0.02)
Pro	1.11 (0.03)	1.18 (0.05)	1.06 (0.05)	1.01 (0.05)
Ser	0.60 (0.02)	0.58 (0.02)	0.57 (0.02)	0.51 (0.02)
Thr	0.37 (0.01)	0.37 (0.01)	0.35 (0.01)	0.33 (0.01)
Tyr	0.41 (0.02)	0.39 (0.01)	0.38 (0.02)	0.34 (0.01)
Val	0.54 (0.02)	0.53 (0.01)	0.51 (0.02)	0.48 (0.02)

*Asx signifies the combined results of aspartic acid and asparagine. **Glx signifies the combined results of glutamic acid and glutamine.

Table A7.2 Amino Acid concentrations (g/100 g (DW) as protein amino acids) in grain (variety Janz) grown within organic and conventional farming systems in low rainfall comparisons (n=8). Values in parentheses are the standard errors.

Amino Acid	Organic	Conventional
Ala	0.41 (0.02)	0.44 (0.02)
Arg	0.65 (0.03)	0.69 (0.03)
Asx*	0.63 (0.02)	0.69 (0.03)
Cys	0.29 (0.01)	0.31 (0.01)
Glx**	4.07 (0.28)	4.34 (0.22)
Gly	0.45 (0.03)	0.49 (0.03)
His	0.34 (0.02)	0.37 (0.02)
Ile	0.51 (0.03)	0.55 (0.03)
Leu	0.88 (0.04)	0.95 (0.05)
Lys	0.27 (0.02)	0.29 (0.02)
Met	0.25 (0.01)	0.26 (0.01)
Phe	0.65 (0.03)	0.71 (0.04)
Pro	1.32 (0.08)	1.41 (0.07)
Ser	0.66 (0.03)	0.72 (0.04)
Thr	0.40 (0.02)	0.43 (0.02)
Tyr	0.45 (0.02)	0.48 (0.03)
Val	0.58 (0.03)	0.62 (0.03)

*Asx signifies the combined results of aspartic acid and asparagine. **Glx signifies the combined results of glutamic acid and glutamine.

Appendix 8

Diet Formulations for the Feeding Trial

Table A8.1 Composition of experimental diets* (adapted from Reeves *et al.*, 1993).

Ingredients (%)	SYN	ORG	ORG+PHYT	CONV	CONV+Zn
Casein	15.0	4.0	4.0	6.1	6.1
Wheat	0	80	80	80	80
Corn Flour	50	0	0	0	0
Sucrose	11.48	0	0	0	0
Alpha-cellulose	5	0	0	0	0
Sunflower Oil	13.50	10.42	10.42	8.46	8.46
Choline Chloride	0.2	0.2	0.2	0.2	0.2
Mineral Mix**	3.5	3.5	3.5	3.5	3.5
Vitamin Mix***	1	1	1	1	1
L-Methionine	0.300	0.385	0.385	0.360	0.360
L-Lysine	0	0.475	0.475	0.360	0.360
Hexatriacontane****	0.02	0.02	0.02	0.02	0.02

*SYN (modified AIN-93G), ORG (Organically grown grain base), ORG+PHYT (Organically grown grain base plus phytate to the level of conventionally grown grain), CONV (Conventionally grown grain base) and CONV+Zn (Conventionally grown grain base plus zinc to the level of organically grown grain). **Mineral mix formulations are included in Tables A8.3-A8.4. ***Vitamin mix formulation from Reeves *et al.*, (1993). Final concentrations are shown in Table A8.5. ****Hexatriacontane is a long chain hydrocarbon included in the diets as a digestability marker.

Table A8.2 Chemical composition of the five rat diets* in terms of protein and limiting amino acids.**

Chemical Composition	SYN	ORG	ORG+PHYT	CONV	CONV+Zn
Protein (%)	12.6	12.0	12.0	12.0	12.0
Lysine (mg/kg)	9750	9670	9670	9645	9645
Methionine (mg/kg)	6450	6370	6370	6363	6363
Threonine (mg/kg)	5025	4380	4380	4444	4444
Arginine (mg/kg)	4800	5760	5760	5792	5792

*Refer to Table A8.1 for diet abbreviations. **Amino acids are calculated based on concentrations in the organically and conventionally grown grain used to formulate the diets and the typical casein analysis reported by Reeves *et al.* (1993).

Table A8.3 Composition (g/kg) of the mineral mix added to the five rat diets* at 35 g/kg (adapted from Reeves *et al.*, 1993).

Ingredient	Source	SYN	ORG	ORG+PHYT	CONV	CONV+Zn
Sodium phytate	Sigma	0.00	0.00	132.30	0.00	0.00
Potassium chloride	Ajax (Univar)	0.00	0.00	300.30	0.00	0.00
Calcium carbonate	Sigma	357.00	332.42	332.42	335.60	335.60
Potassium phosphate	Ajax (Univar)	241.00	116.70	0.00	25.00	25.00
Potassium carbonate	Ajax (Univar)	22.50	219.00	0.00	231.50	231.50
Sodium chloride	Sigma	74.00	235.50	0.00	235.50	235.50
Potassium sulphate	Sigma	46.60	0.00	0.00	36.00	36.00
Magnesium oxide	BDH	24.00	3.20	3.20	0.00	0.00
Ferric citrate	Sigma	6.06	2.79	2.79	2.37	2.37
Zinc carbonate	Ajax (Unilab)	1.63	0.00	0.00	0.00	0.49
Manganous carbonate	Ajax (Unilab)	0.63	0.14	0.14	0.00	0.00
Cupric carbonate	Ajax (Unilab)	0.30	0.14	0.14	0.18	0.18

Table A8.3 (cont.) Composition (g/kg) of the mineral mix added to the five rat diets* at 35 g/kg (adapted from Reeves *et al.*, 1993).

Ingredient	Source	SYN	ORG	ORG+PHYT	CONV	CONV+Zn
Potassium iodate	ANALAR	0.01	0.01	0.01	0.01	0.01
Sodium selenate	Unknown	0.01	0.01	0.01	0.01	0.01
Ammonium molybdate	Sigma	0.01	0.01	0.01	0.01	0.01
Sodium meta-silicate	ACE Chemicals	1.45	1.45	1.45	1.45	1.45
Chromium potassium sulphate	Ajax (Univar)	0.28	0.28	0.28	0.28	0.28
Lithium chloride	Sigma	0.02	0.02	0.02	0.02	0.02
Boric acid	Sigma	0.08	0.08	0.08	0.08	0.08
Sodium fluoride	ANALAR	0.06	0.06	0.06	0.06	0.06
Nickel carbonate	ACE Chemicals	0.03	0.03	0.03	0.03	0.03
Ammonium vanadate	Merck	0.01	0.01	0.01	0.01	0.01
Sucrose (Cane Sugar)	CSR	224.33	88.15	226.75	131.90	131.41

*Refer to Table A8.1 for diet abbreviations.

Table A8.4 Final mineral concentrations (mg/kg) in the five rat diets*, obtained following the mineral mix formulation in Table A8.3. Phytate, Ca, Mg, Na, K, P, S, Fe, Mn, Zn and Cu were also contributed through wheat in the diets. Added casein contributed 35 mg Zn and 7900 mg P/kg casein.

Element	SYN	ORG	ORG+PHYT	CONV	CONV+Zn
Phytate	0	4334	7582	7583	7583
Ca	5003	4995	4995	4995	4995
P	3105	3206	3208	3221	3221
K	3601	9231	9232	9242	9242
S	300	1218	1218	1218	1218
Na	1019	3259	3265	3260	3260
Cl	1571	5000	4999	5000	5000
Mg	507	942	942	942	942
Fe	35.0	34.5	34.5	34.5	34.5
Zn	35.0	23.0	23.0	14.1	23.1
Mn	10.5	18.3	18.3	18.4	18.4
Cu	6.0	6.0	6.0	6.0	6.0
I	0.2	0.2	0.2	0.2	0.2
Mo	0.15	0.15	0.15	0.15	0.15
Se	0.15	0.15	0.15	0.15	0.15
Si	5.0	5.0	5.0	5.0	5.0
Cr	1.0	1.0	1.0	1.0	1.0
F	1.0	1.0	1.0	1.0	1.0
Ni	0.5	0.5	0.5	0.5	0.5
B	0.5	0.5	0.5	0.5	0.5
Li	0.10	0.10	0.10	0.10	0.10
V	0.10	0.10	0.10	0.10	0.10

*Refer to Table A8.1 for diet abbreviations.

Table A8.5 Vitamin concentrations within the five rat diets (based on Reeves *et al.*, 1993).

Vitamin	U	U/kg diet
Nicotinic acid	mg	30
Pantothenate	mg	15
Pyridoxine	mg	6
Thiamin	mg	5
Riboflavin	mg	6
Folic acid	mg	2
Vitamin K	ug	750
D-Biotin	ug	200
Vitamin B-12	ug	25
Vitamin A	IU	4000
Vitamin D3	IU	1000
Vitamin E	IU	75

Appendix 9

Details of Sampling and Analyses Associated with the Rat Feeding Trial

A9.1 Breath Sampling

Breath samples were collected by placing the rat unrestrained in an enclosed chamber for 5 minutes. Air entering the chamber was filtered through a soda lime column as a carbon dioxide trap and air in the chamber was circulated through a calcium chloride column (moisture trap) so that relative humidity was maintained at a constant level (refer to Figure A9.1). After 5 minutes, the chamber was isolated completely and approximately 20 ml of air removed for analysis using a syringe.

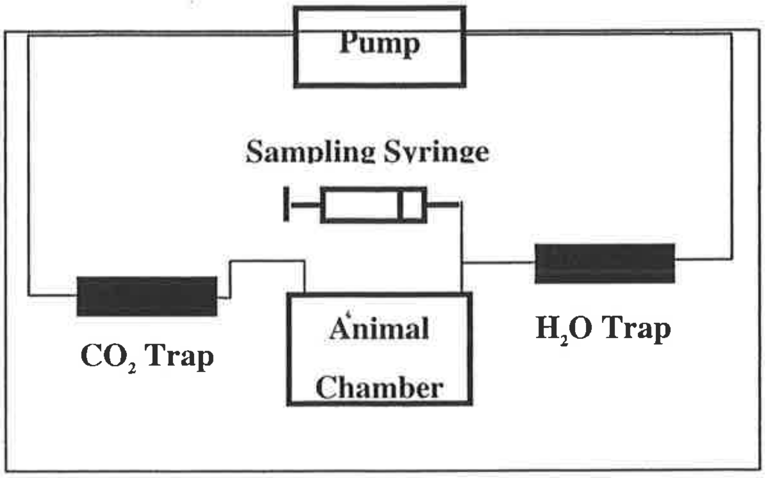


Figure A9.1 The closed circuit breath sampling system used for collecting rat breath for hydrogen, methane and carbon dioxide analysis. Figure taken from Porter *et al.* (1998).

A9.2 Elemental Analysis of Urine, Faeces, Blood Plasma, Liver, Muscle and Bone

The equivalent of 1.5 ml undiluted urine, diluted to 5 ml with milli-Q water, was left overnight in 4 ml concentrated nitric acid. The following day it was digested (adapted from Zarcinas *et al.*, 1987) for 25 minutes at 80 ˚C, 30 minutes at 100 ˚C, 90 minutes at 120 ˚C, 60 minutes at 125 ˚C, 60 minutes at 135 ˚C and finished off at 141 ˚C until 0.5 ml of solution remained.

Dried and ground faeces (0.5 g) were left overnight in 6 ml concentrated nitric acid and digested (based on Zarcinas *et al.*, 1987) for 25 minutes at 80 ˚C, 20 minutes at 100 ˚C, 90 minutes at 125 ˚C and finished off at 140 ˚C until 1 ml of solution remained (approximately 170 minutes). In order to maximise iron recovery, dried and ground faeces samples were also left overnight and then digested in 10 ml of nitric acid and 1 ml of perchloric acid for 20 minutes at 80 °C, 20 minutes at 100 °C, 20 minutes at 120 °C, 60 minutes at 130 °C, 30 minutes at 140 °C, 50 minutes at 150°C, 40 minutes at 170°C, 15 minutes at 180 C and 7 minutes at 225 °C.

Blood plasma (0.1 ml) was left overnight in 4 ml concentrated nitric acid. The digestion program was the same as that for the urine.

Liver and muscle samples (0.4 g dry weight) were left overnight in 6 ml concentrated nitric acid and then digested for 25 minutes at 80 °C, 20 minutes at 100 °C, 90 minutes at 125 °C and then at 140 °C until only 1 ml of solution remained. This first digestion was in order to break down the fats in these tissues. The remaining solution was then digested in 10 ml of nitric acid and 1 ml of perchloric acid using the same digestion program used for the faeces (nitric and perchloric acids).

Whole femurs (dried and residual flesh removed with ethanol) were left overnight in 6 ml concentrated nitric acid. The digestion program was the same as that for the faeces (nitric acid).

The digested samples were cooled and diluted to either 10 ml (blood plasma samples) or 25 ml (urine, faeces and bone samples) using 1% nitric acid. They were analysed for iron, manganese, copper, zinc, calcium, magnesium, sodium, potassium, phosphorus and sulphur concentrations using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES). The machine used was an ARL 3580 B ICP with the plasma in radial mode and using liquid nebulisation.

Appendix 10

Consumption, Excretion and Growth Data from the Rat Feeding Trial

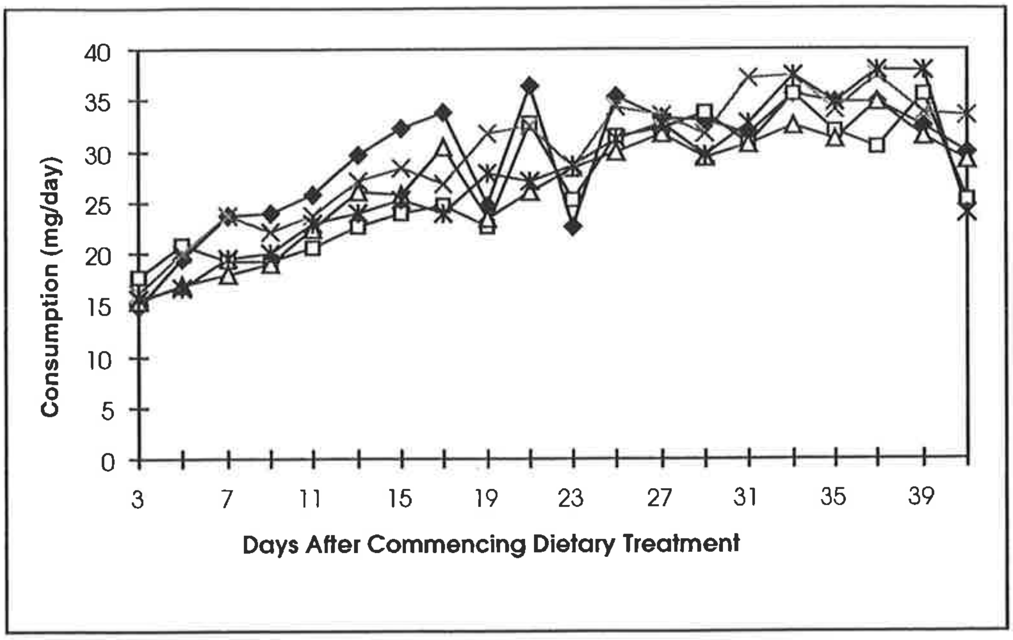


Figure A10.1 Water consumption of rats fed organic (ORG, ♦), organic with phytate added to the level of conventional (ORG+PHYT, □), conventional (CONV, Δ), conventional with zinc added to the level of organic (CONV+Zn, ×) or a synthetic AIN-93G modified (SYN, ✶) diet over 42 days of dietary treatment. When comparing treatments within the same time point, the standard error of difference is 6.09.

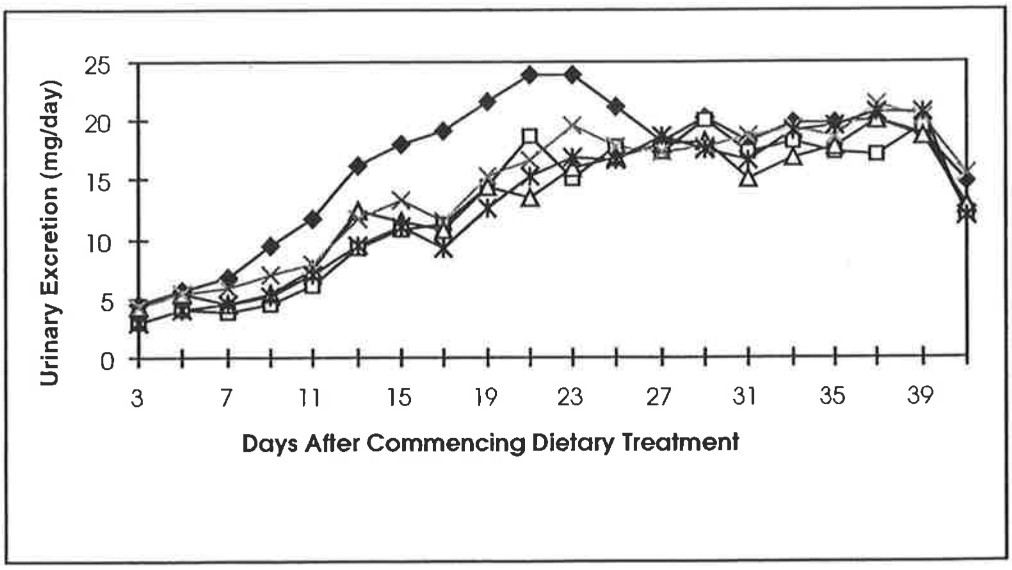


Figure A10.2 Urinary excretion by rats over 42 days of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✶) diets (abbreviations defined in Figure A10.1). When comparing treatments within the same time point, the standard error of difference is 5.45.

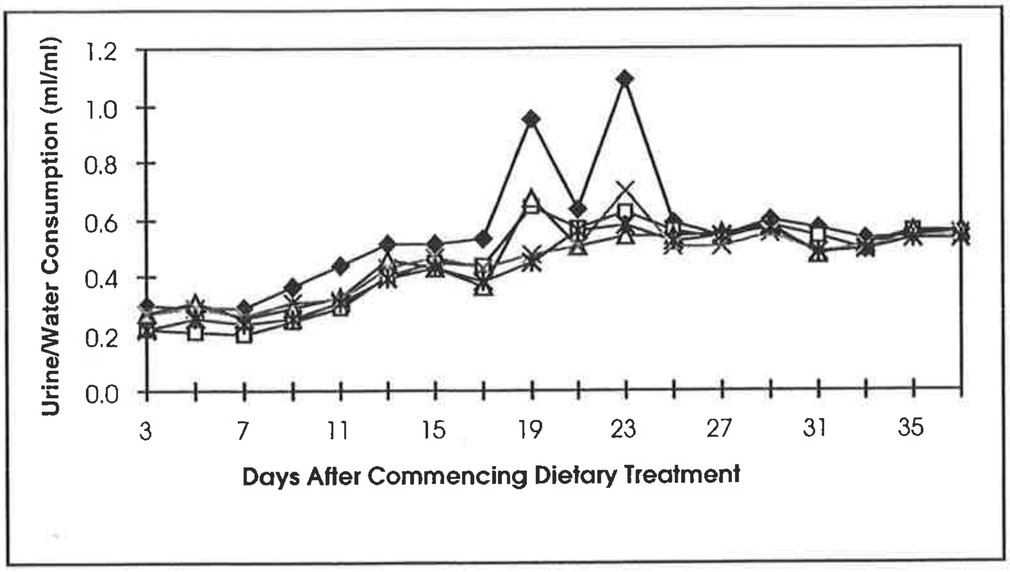


Figure A10.3 Urine excreted per ml of water consumed by rats over 42 days of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✱) diets (abbreviations defined in Figure A10.1). When comparing treatments within the same time point, the standard error of difference is 0.126.

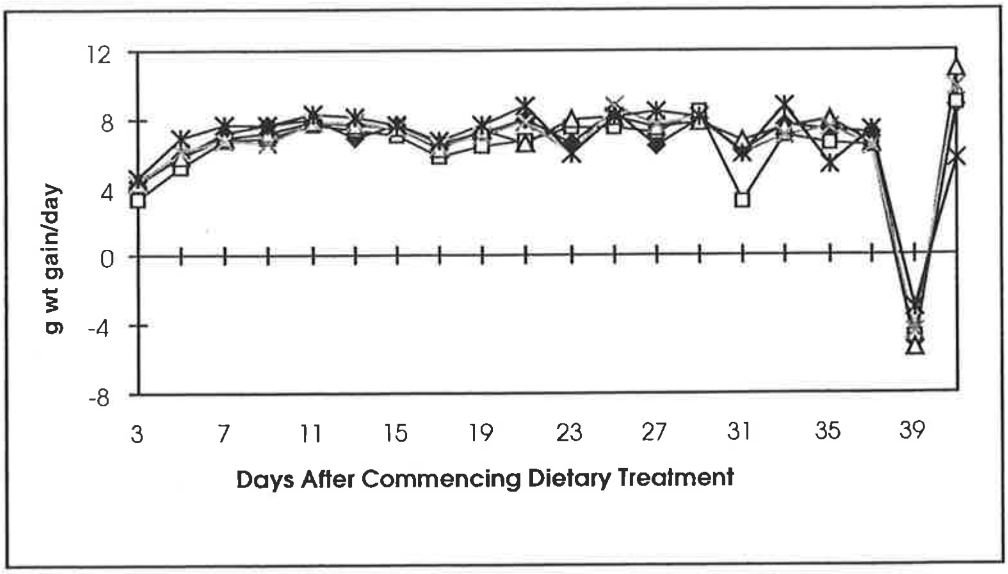


Figure A10.4 The weight gain per day of rats over 42 days of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✱) diets (abbreviations defined in Figure A10.1). When comparing treatments within the same time point, the standard error of difference is 2.05.

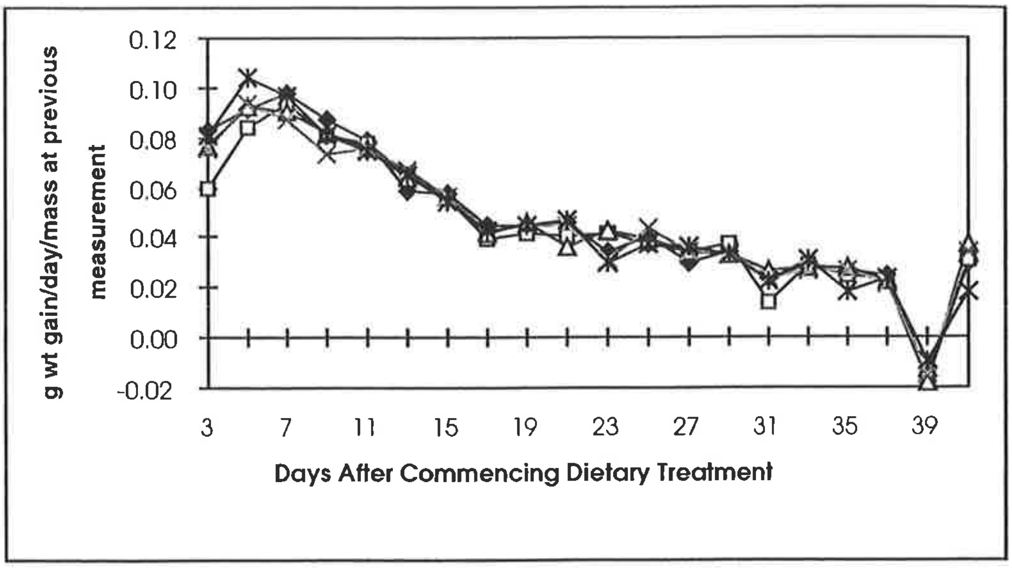


Figure A10.5 The relative weight gain (weight gain adjusted for weight at the previous measurement) of rats over 42 days of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✳) diets (abbreviations defined in Figure A10.1). When comparing treatments within the same time point, the standard error of difference is 0.0178.

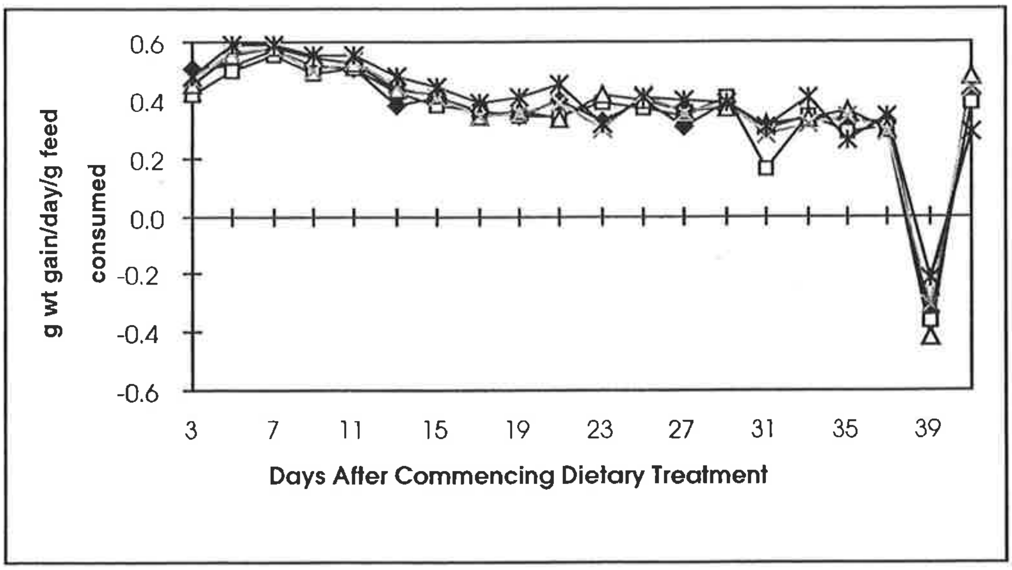


Figure A10.6 The feed efficiency ratios (weight gain per feed consumed) for rats over 42 days of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✳) diets (abbreviations defined in Figure A10.1). When comparing treatments within the same time point, the standard error of difference is 0.127.

Appendix 11

Breath Analysis

A11.1 Introduction

Breath analysis is a useful tool because the gases present in the breath are the gaseous excreta of metabolic functions (for example, ethane and pentane as products of inflammation) and the bacteria present in the gastrointestinal tract (for example, hydrogen and methane produced by *Bifidobacteria* and methanogenic bacteria respectively). The most common clinical uses of breath analysis are the detection of infection by *Helicobacter pylori* and the diagnosis of lactose intolerance. Other uses of breath analysis, including the determination of intestinal permeability and assessment of inflammatory response, are still being developed and, therefore, not usually adopted by clinicians.

This study utilises breath analysis to determine the transit time of substrate within the gastrointestinal tract. This is possible because the exhalation of labelled carbon dioxide following a meal including readily digestible substrate with labelled carbon, indicates movement of the substrate through the small intestine. The exhalation of hydrogen and methane indicate that indigestible substrate is moving through the colon where it is digested by the colonic bacteria.

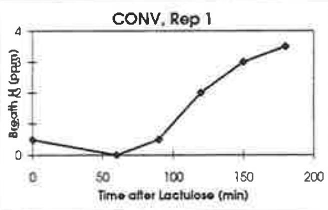
This study also uses breath analysis as an indication of the digestibility of the treatment diets, the production of hydrogen or methane occurring only after malabsorption in the small intestine. Furthermore, the hydrogen and methane profiles are indicative of the species of bacteria present in the colon and is used here to compare the prebiotic effects (that is, the effects of food on the bacterial populations in the colon) of the dietary treatments.

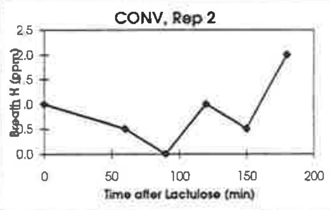
A11.2 Hydrogen and Methane Production

A11.2.1 Results

The progressive hydrogen production of individual rats on day 39 is shown in Figure A11.1. The extremely high outlier peaks at 60 minutes after the lactulose meal for CONV rep 5, CONV+Zn rep 4, ORG+PHYT rep 4, SYN rep 4 and 120 minutes after the lactulose meal for ORG rep 2 are characteristic of bacteria present in the stomach or small intestine due to copraphagy and these points have therefore been excluded from the hydrogen data set for other calculations.

Figure A11.1 Progressive hydrogen production by rats fed a lactulose meal following a 12 hour fast, 39 days after the initiation of dietary treatments: ORG (organically grown grain base), ORG+PHYT (organically grown grain base plus phytate to the level of conventionally grown grain), CONV (conventionally grown grain base), CONV+Zn (conventionally grown grain base plus zinc to the level of organically grown grain) and SYN (modified AIN-93G).


CONV, Rep 1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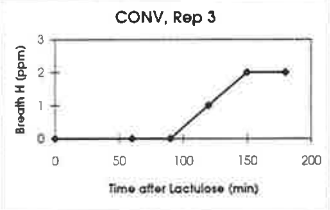
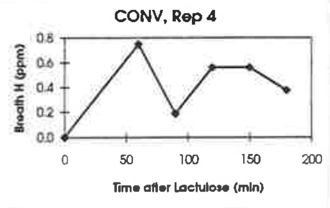

CONV, Rep 3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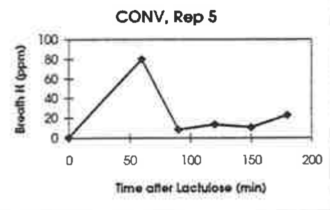
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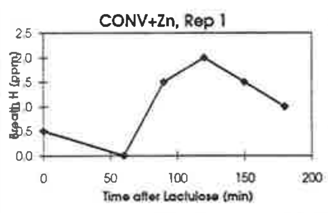
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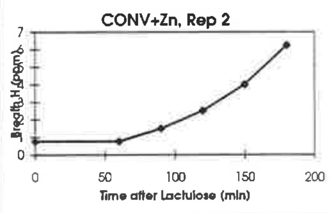
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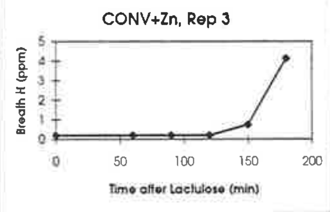
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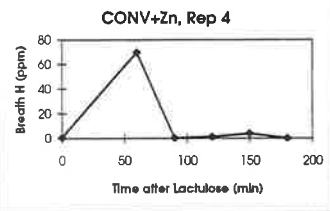
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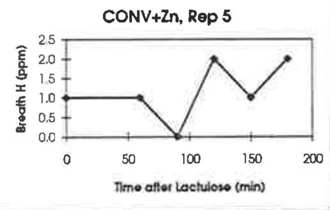
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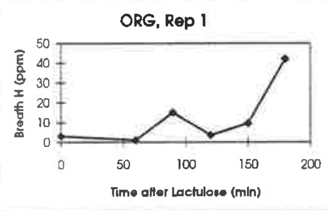
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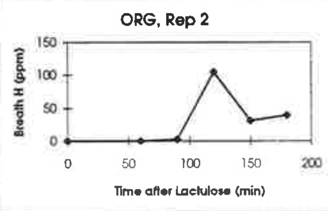
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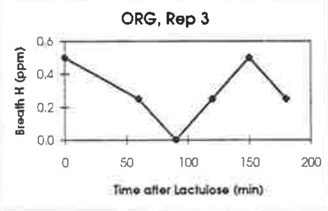
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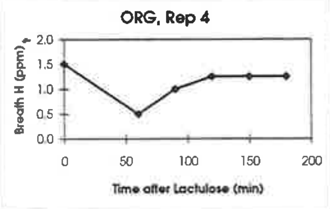
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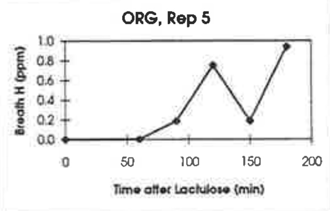
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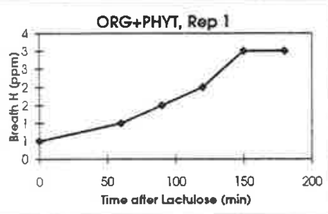
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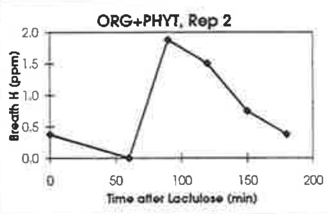
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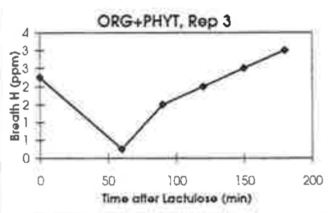
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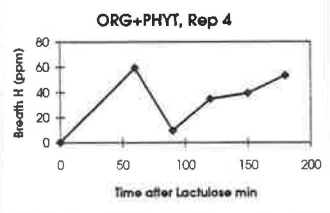
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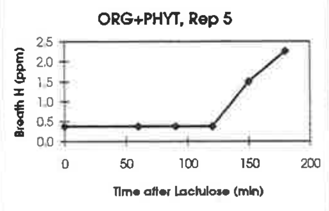
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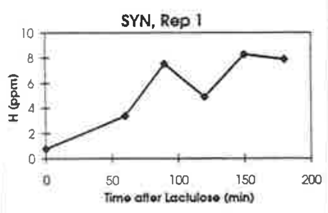
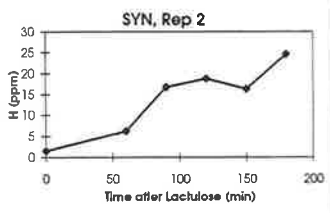

SYN, Rep 1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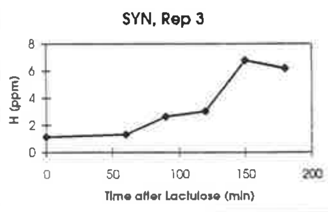
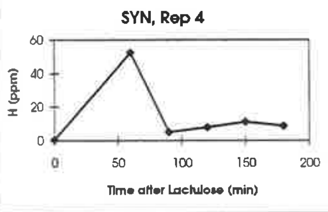

SYN, Rep 3


SYN, Rep 4

On day 30 of the experiment, there was no significant difference in breath hydrogen or methane between the dietary treatments (Table A11.1). Likewise, on day 39 the rats fed different diets produced statistically similar amounts of breath hydrogen (Figure A11.2) and methane (Figure A11.3) except at the measurement made 60 minutes after feeding the lactulose meal. At this time, the rats fed the SYN diet produced significantly greater hydrogen than those fed the other diets. On day 42, the rats fed the CONV diet produced significantly greater hydrogen than those fed the CONV+Zn and the SYN diets (Table A11.1). The rats fed the other diets were intermediate in hydrogen production and statistically similar to both the CONV, CONV+Zn and SYN diets on day 42. Similar to day 30, there was no significant difference on day 42 between the dietary treatments in terms of methane production (Table A11.1).

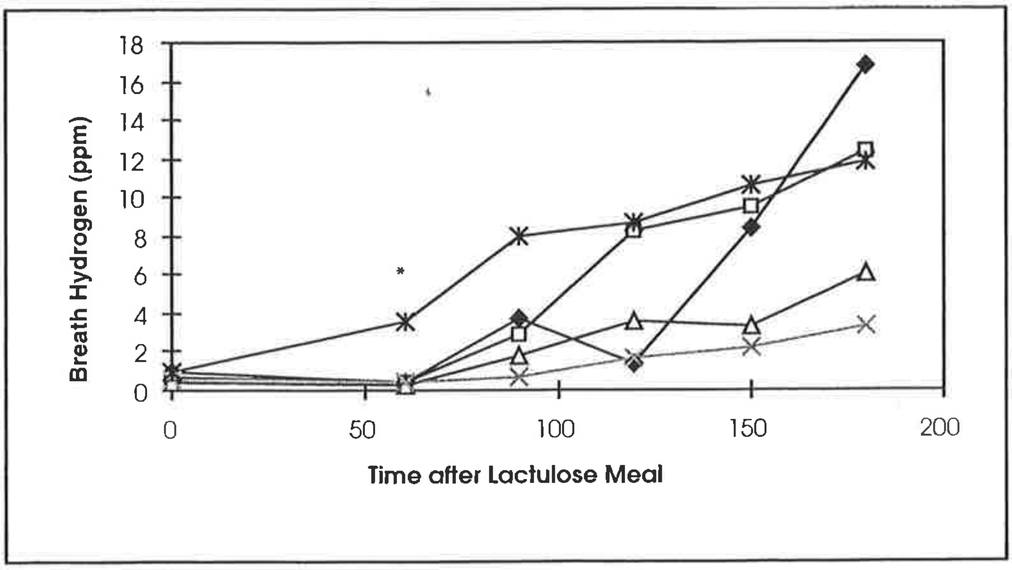


Figure A11.2 Breath hydrogen concentration in rats fed a lactulose meal following a 12 hour fast, 39 days after the initiation of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✶) diets (abbreviations defined in Figure A11.1). *The SYN diet is significantly greater than the other diets ($P<0.05$). There were no significant differences between diets at all other time points.

Table A11.1 Hydrogen (data transformed) and methane concentration in breath of rats, 30 or 42 days after commencing dietary treatments*.

	SYN	ORG	ORG+PHYT	CONV	CONV+Zn	F Prob
Hydrogen (ppm)						
Day 30**	6.13*** (37.58)	7.38 (54.46)	6.17 (38.07)	3.5 (12.25)	4.8 (23.04)	NS P=0.066
Day 42**	1.62^{a} (2.62)	3.56^{ab} (12.67)	3.46^{ab} (11.97)	6.41^{b} (41.09)	2.87^{a} (8.24)	P=0.009
Methane (ppm)						
Day 30	4.00***	0.80	1.00	1.80	0.60	NS P=0.062
Day 42	0.04	0.04	0.00	0.08	0.08	NS

*Abbreviations defined in Figure A11.1. **The data for the hydrogen measurements was square root transformed during statistical analysis. Values in parantheses are back transformed for ease of interpretation. ***There is a significant difference between days 30 and 42 within this dietary treatment. ^{ab}Means within the same row followed by the same lower case letter are not significantly different.

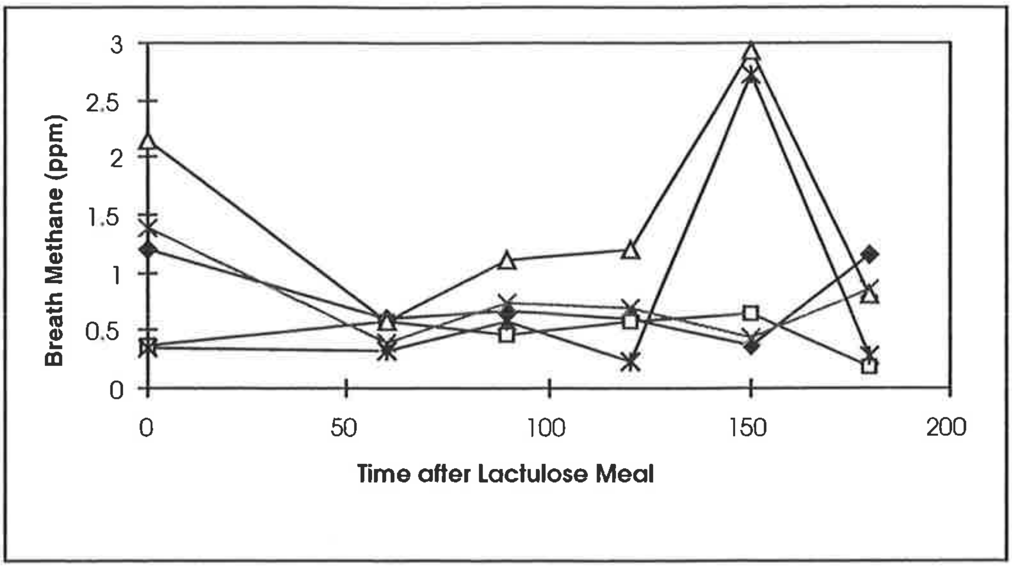


Figure A11.3 Progressive methane production by rats fed a lactulose meal following a 12 hour fast, 39 days after the initiation of treatment with ORG (♦), ORG+PHYT (□), CONV (Δ), CONV+Zn (×) or SYN (✳) diets (abbreviations defined in Figure A11.1). There were no significant differences between diets at any time points.

When the rats were grouped into ORG(+) and CONV(+) and compared to SYN the result was different: On day 30, rats fed CONV(+) diets produced significantly less breath hydrogen than those fed ORG(+) diets and rats fed the SYN diet were intermediate in hydrogen production and statistically similar to both ORG(+) and CONV(+) (Table A11.2). On day 42, the SYN fed rats appeared to produce lower breath hydrogen than those fed CONV(+) diets (Table A11.2).

When the breath hydrogen production at day 30 is compared with that at day 42, following the fast and lactulose meal, the rats fed the SYN and ORG(+) diets are producing significantly less hydrogen at day 42 whereas the opposing non-significant trends in the CONV and CONV+Zn dietary treatments result in the CONV(+) fed rats producing statistically similar amounts of hydrogen between the two measurements (Table A11.2).

Table A11.2 Hydrogen (data transformed) and methane concentration in breath of rats, 30 or 42 days after commencing dietary treatments*.

	SYN	ORG(+)	CONV(+)	F Prob
Hydrogen (ppm)				
Day 30**	6.13^{ab***} (37.58)	6.78^{b***} (45.97)	4.15^{a} (17.22)	P=0.026
Day 42**	1.62 (2.62)	3.52 (12.39)	4.64 (21.53)	NS P=0.053
Methane (ppm)				
Day 30	4.00^{b***}	0.90^{a}	1.20^{a}	P=0.015
Day 42	0.04	0.02	0.08	NS

*Dietary treatments grouped by basal grain source as defined in Figure A11.4. **The data for the hydrogen measurements was square root transformed during statistical analysis. Values in parantheses are back transformed for ease of interpretation. ***There is a significant difference between days 30 and 42 within this dietary treatment. ^{ab}Means within the same row followed by the same lower case letter are not significantly different.

Methane production on day 30 was significantly greater by those rats fed the SYN diet than the rats fed the ORG(+) and CONV(+) diets . However, on day 42 there was no significant difference between the diets in terms of methane production (Table A11.2). Comparing the day 30 and day 42 results, reveals that the rats fed the SYN diet were producing significantly less methane on day 42 (Table A11.2).

At day 39, following the lactulose meal, there was no significant effect of basal grain source on breath hydrogen or methane at any of the time points except at 60 minutes post feeding when the SYN fed rats had significantly greater breath hydrogen than both ORG(+) and CONV(+) fed rats (Figure A11.4).

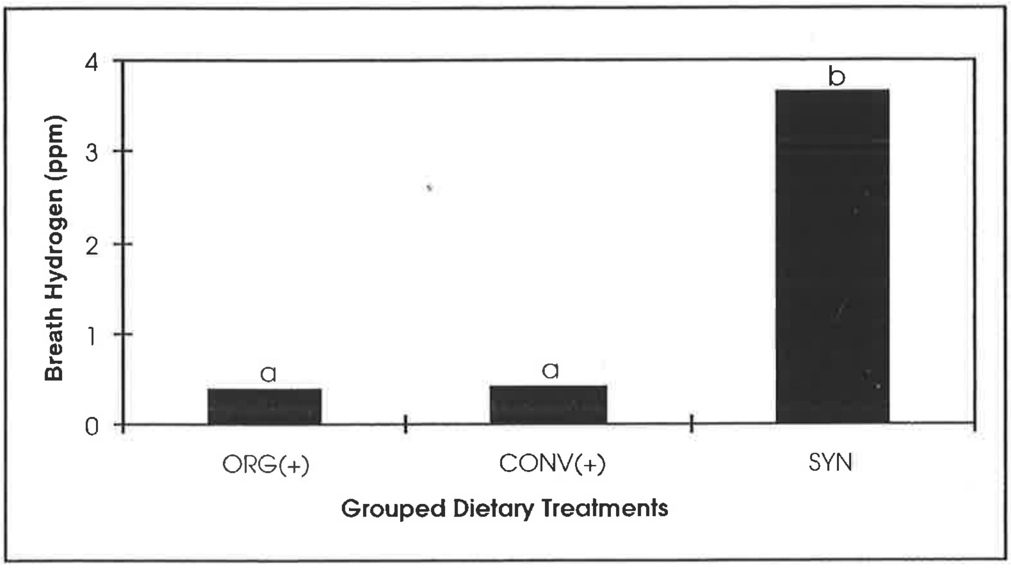


Figure A11.4 Breath hydrogen concentrations of rats fed diets based on organically (ORG(+)) or conventionally (CONV(+)) grown grain or the AIN-93G formulation (SYN) with the substrate being lactulose administered after a 12 hour fast. Measurements were taken 60 mins after the lactulose meal. Treaments with the same lower case letter are not significantly different ($P<0.05$).

A11.2.2 Discussion

Breath samples taken on days 30 and 42 were each showing small windows of gas production. Because rats were feeding *ad libitum* on these days, these measurements include substrate moving through various parts of the digestive tract. On day 39, however, the measurements accurately reflect the passage of substrate through the digestive tract over time and, although the substrate fed here is not the same substrate that has helped to determine the composition of the intestinal micro-flora, the lactulose can help to identify differences in metabolic activity that have developed over 5 weeks of feeding on the treatment diets.

The breath hydrogen and methane concentrations, on day 30, of rats fed the different diets were only significantly different when the diets were grouped by the basal grain source of the diet (Tables A11.1 and A11.2). This indicates that phytate and zinc are probably not major factors involved in this effect. The fact that the ORG(+) and CONV(+) groups are significantly different here is important because it indicates that there are differences between these two grains, besides the zinc and phytate. These unelucidated differences are either causing different populations of micro-flora (for example, the ORG(+) fed rats sustaining more hydrogen producers and the SYN fed rats sustaining more methane producers) or causing different quantities of substrate to reach the colon. This could mean that the grains differ in their prebiotic effects. When the same substrate was used for all rats on day 39, the rats fed ORG(+) and CONV(+) diets had similar hydrogen and methane production (except for hydrogen production 60 minutes after the meal). This observation refutes the idea of the dietary treatments effecting microbial populations and supports the malabsorption hypothesis. A discussion of possible compositional differences within the carbohydrate and fibre fractions of the grains is presented in Chapter 5 (section 5.4.2)

An alternative explanation of why the CONV(+) fed rats produced less hydrogen than the ORG(+) fed rats and why the SYN fed rats produced greater methane than both ORG(+) and CONV(+) fed rats when measured on day 30 is that possibly they were feeding by different patterns. Rats are nocturnal and have been shown under *ad libitum* conditions to consume the majority of their food in the dark (Williams *et al.*, 2000). If, however, the SYN, ORG(+) and CONV(+) fed rats were eating at slightly different times, less substrate may have been present in the colon (ie. being metabolised to hydrogen and methane by colonic bacteria) at the point at which the measurements were taken. A similar argument could be used in explaining the contrast between day 30 and day 42 measurements. This explanation is unlikely but would also result in ORG(+) and CONV(+) fed rats producing similar amounts of hydrogen at each time point after ingestion as was measured on day 39.

With the lactulose substrate, the only difference in breath hydrogen between the dietary treatments is that there is significantly greater hydrogen produced by rats fed the SYN diet at 60 minutes after ingestion of lactulose (Figure A11.2). This is probably due to the SYN diet altering the motility of the gastrointestinal tract (resulting in faster passage of digesta (refer to Chapter 5, Table 5.8)) and this was carried over to the lactulose test. This would result in substrate reaching the colon earlier in those rats usually fed the SYN diet than those usually fed the grain based diets. It also points towards the fact that the faster transit time of the SYN diet was a physiological adaptation to the diet, not occurring only when the SYN diet was the substrate. The same effect may not have been observed in the methane profile because the methanogenic bacteria may have been localised further along the colon as was found in methane producers by Macfarlane *et al.* (1992) and Pochart *et al.* (1993).

The breath samples taken at days 30 and 39 can be considered as showing real effects of the dietary treatments whereas the breath samples from day 42, which showed quite contrasting results for those rats fed the SYN diet, may actually still be showing effects of the overnight fast (only 2-3 days earlier) or the lactulose meal. Because lactulose is a prebiotic probe, it may alter the bacterial populations within the colon. It is useful, however, because it provides an indication of the bacterial populations present in the colon at the time of the lactulose meal and differences between these populations (assuming they are consistent within treatments) would be due solely to diet. The prebiotic effect of lactulose, however, may take time to equilibrate once the rats return to their treatment diets and the bacterial populations may not yet have reverted to pre-lactulose levels at the time measurements were taken at day 42.

When refed after the fast, the rats would possibly eat out of cycle (ie. not at their usual nocturnal feeding time) in response to hunger. This could mean that the cyclic nature of bacterial population growth in the colon was interrupted and had not yet regained equilibrium at day 42.

Fasting has been said to have no effect on intestinal microflora because no difference was found in breath hydrogen production between rats fasted for 12 or 72 hours (Griffin *et al.*, 1984). However, in that experiment both the control and experimental animals were fasted (necessary in order to effectively use the lactulose probe) and all were raised on the same diet. Therefore, fasting may have an effect in the current experiment if the treatment diets differed in their prebiotic capacity, which is possible as indicated by the breath hydrogen and methane production on day 30 (Table A11.2).

It has been shown for mice (Burholt *et al.*, 1985) that epithelial cell proliferation in the digestive tract follows a circadian rhythm which was phase shifted by feeding the mice at a time when they were not usually eating. It was also shown that during a fast, cell proliferation within the colon dropped dramatically and did not continue within the circadian rhythm (Burholt *et al.*, 1985). Refeeding during the light cycle (mice, like rats, feed mostly at night) after a 48 hour fast, resulted in the cell proliferation in the colon being out of synchronisation with the original circadian rhythm. After three to four days following the initial refeeding, cell proliferation in the colon approached the original circadian rhythm but was still peaking slightly later. Increased epitheliel cell proliferation is likely to be accompanied by an increase in sloughed epitheliel cells. These sloughed cells are part of the available substrate for bacteria in the colon (Forsberg *et al.*, 1997). Therefore, the reduction of this substrate during fasting may alter some bacterial populations in the colon and these could be expected to approach pre-fasting populations only when cell proliferation, and hence sloughing, becomes in phase with the original circadian rhythm.

The important point here is that when breath measurements were taken on day 42, it is quite possible, considering the 3-4 day equilibration time for cell proliferation following a fast, that the bacterial populations in the colon were still equilibrating after the fast and lactulose meal 2-3 days earlier.

Interestingly, the rats fed the SYN diet produced significantly less hydrogen on day 42 than day 30. There was also a trend towards the rats fed the ORG, ORG+PHYT and CONV+Zn diets to have lower breath hydrogen production on day 42 (following the fast) than they did on day 30. This may indicate that the CONV diet is contributing to a faster bacterial equilibration in the caecum following an upset (either fasting or a lactulose meal) than the other diets investigated. Alternatively, the trend toward higher breath hydrogen on day 42 in the rats fed the CONV diet suggests that the CONV diet may support greater populations of hydrogen consuming bacteria in the colon than the other diets and these have not yet reached equilibrium either.

The CONV diet differed from the CONV+Zn diet only in its lower zinc content and all of the other diets had zinc levels similar to the CONV+Zn diet. This may implicate zinc in either slowing down bacterial equilibration or in producing conditions favourable for the hydrogen producers rather than the hydrogen-consuming bacteria in the colon. However, further validation is required.

The rats fed the SYN diet also produced significantly less methane on day 42 compared with day 30 of the experiment. This result may be an indication of differences in microbial populations between the dietary treatments, with the SYN diet resulting in greater methane producing bacteria. If these bacteria do not break down lactulose, the fast followed by the lactulose meal would have resulted in reducing the methanogen populations which may not have regained equilibrium by day 42.

It is important to note that hydrogen and methane are not the only products of colonic bacteria albeit the only ones measured in this study. There are also colonic bacteria which produce small chain fatty acids (SCFA's), for example acetate, butyrate and propionate. Therefore, the dietary treatments could be eliciting changes in the profile of these volatile compounds which have not been detected in this study.

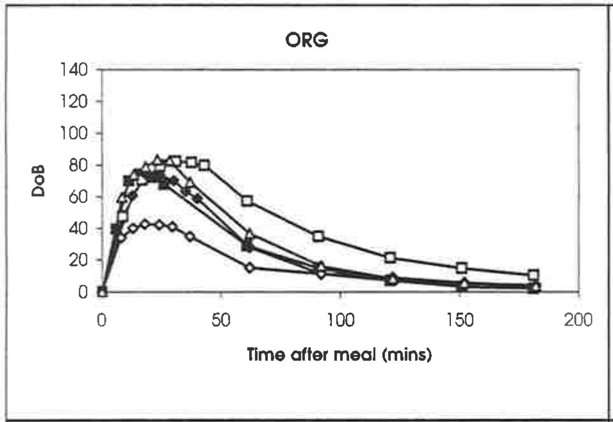
The differences in hydrogen and methane production observed in this study could also be due to differences in intestinal environment, possibly induced by dietary treatments. One example of such a change is in the metabolism of the animal, such that the rate at which hydrogen is moved into the blood stream and lungs is different between the treatments. For the data reported here, however, the metabolic potential of the rats was not significantly associated with hydrogen production (it was not statistically significant as a covariate). Another possible change in intestinal environment is in the luminal pH. In this case, hydrogen may be taken out of the lumen in order to buffer the pH. Hydrogen used in this way is not absorbed into the blood stream and exhaled in the breath.

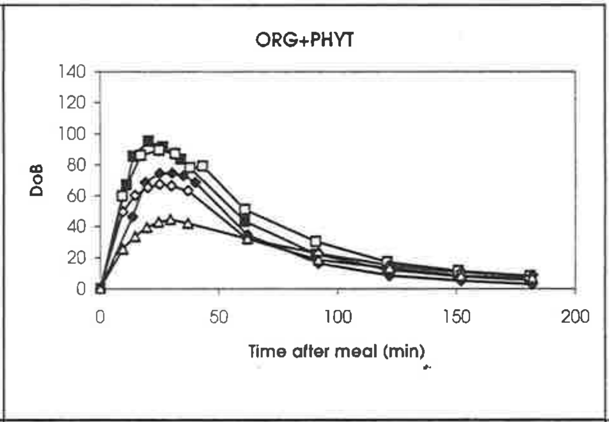
Some researchers in the past have questioned the reproducibility of breath tests. Certainly, the variation between individuals can be very great (Gelissen *et al.*, 1994; Hughes *et al.*, in press) but variation within individuals is thought to be less and therefore individuals are commonly used as their own control within studies where breath tests are used (for example, Basilisco *et al.*, 1985; Amann *et al.*, 1998; Peuhkuri *et al.*, 1999). As a validation for this technique, (Gelissen *et al.*, 1994; Hughes *et al.*, in press) found that most individuals ranked similarly in hydrogen production over two measurements regardless of quantitative changes in hydrogen production. A significant correlation between two replicate measurements of breath hydrogen was also found by Casellas and Malagelada (1998) when testing the reproducibility of different substrates.

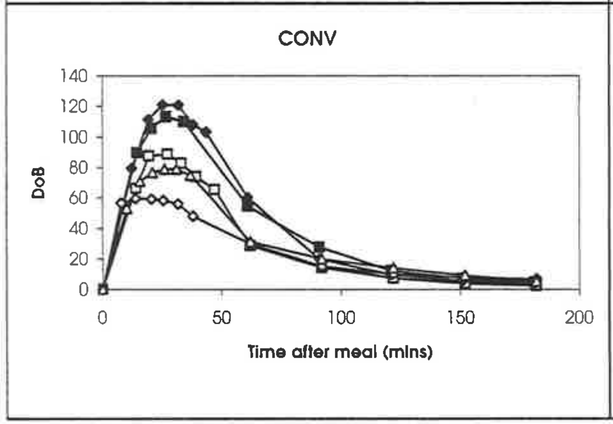
A11.3 Isotopic Carbon Dioxide Production

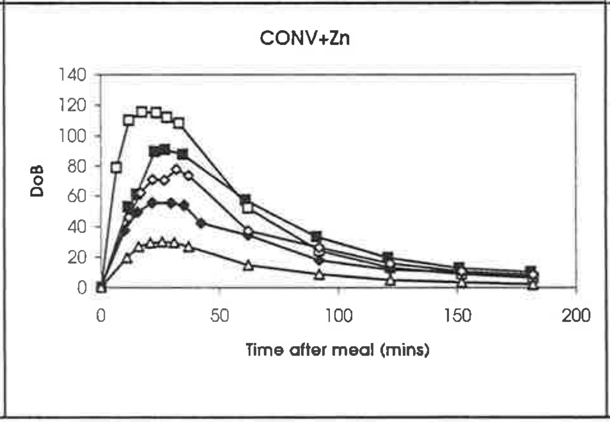
The presence of ^{13}C labelled carbon dioxide in the breath is indicative of the passage of the ^{13}C labelled octanoic acid through the gut. Graphs of the change in isotopic ratios in the breath (Figure A11.5) were used to calculate the rate of passage of substrate through the gastric phase of the gastrointestinal tract (expressed as gastric half emptying time in Table 5.8).

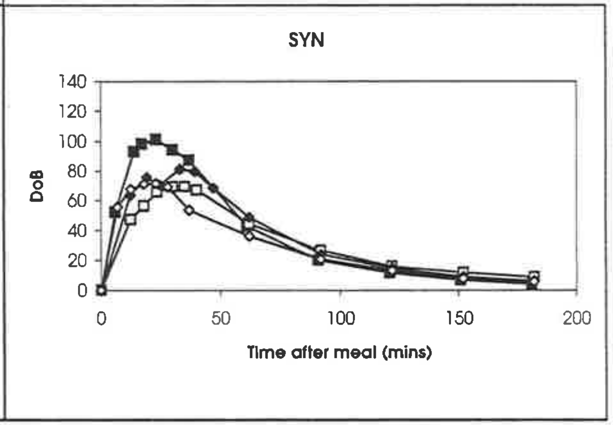
Figure A11.5 The progressive exhalation of ^{13}C as ^{13}CO2 by individual rats (Rep 1 ■; Rep 2 □; Rep 3 ♦; Rep 4 ◊; Rep 5 Δ) after consuming a lactulose meal spiked with ^{13}C octanoic acid 39 days after the commencement of dietary treatments (abbreviations defined in Figure A11.1).


ORG
DoB
140
120
100
80
60
40
20
0
0
50
100
150
200
Time after meal (mins)


ORG+PHYT
DoB
140
120
100
80
60
40
20
0
0
50
100
150
200
Time after meal (min)


CONV
DoB
140
120
100
80
60
40
20
0
0
50
100
150
200
Time after meal (mins)


CONV+Zn
DoB
140
120
100
80
60
40
20
0
0
50
100
150
200
Time after meal (mins)


SYN
DoB
140
120
100
80
60
40
20
0
0
50
100
150
200
Time after meal (mins)

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