THE EFFECT OF GENOTYPE, DRYING CONDITIONS AND MICROBIAL ENZYME SUPPLEMENTATION ON THE NUTRITIVE VALUE OF HYBRID AND OPEN POLLINATED MAIZE GRAIN FOR BROILER FEEDING.

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I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text.

K. C. E. Khumalo
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ABSTRACT

Three experiments were conducted to examine the effects of tannin inclusion in the diet; heat treatment of maize; and maize genotype and sex of broilers; on the nutritive value of maize-soyabean based diets, as affected by dietary enzyme supplementation. In Experiment 1, six hundred day-old Ross male chicks were given 5 main diets. The 5 main diets were based on hybrid yellow maize and a commercial soyabean concentrate and supplemented with tannin at 0, 5, 15, 20 or 25 g/kg. In addition each diet was either supplemented with Avizyme 1502 (Finnfeeds International, UK) at 1 g/kg diet, or not. The birds were given experimental feed from day one to day 22 and the experiment was a completely randomised design with 5x2 factorial arrangement of treatments, and 6 replicates. The inclusion of tannin in the diet significantly reduced feed intake ($P<0.000$), weight gain ($P<0.000$) and feed conversion efficiency. The feed conversion efficiency followed a curvilinear response to dietary tannin level, from a low at the lowest tannin level, to a peak at tannin level of between 10 and 15 g/kg, whereafter it started declining with further increase in dietary tannin. Tannin inclusion also significantly reduced the digestibility of protein ($P<0.01$), gross energy ($P<0.05$), and phosphorus ($P<0.05$). A similar trend was seen in terms of ileal digestibility of amino acids. The jejunum tissue protein was significantly reduced due to tannin inclusion in the diet. The activity of the digestive enzymes was not significantly affected by tannin inclusion in the diet. Avizyme 1502 supplementation in the diet significantly ($P<0.05$) improved weight gain of the birds. There was a non-significant increase in the food intake and feed conversion efficiency of broilers on diets containing tannin, due to Avizyme 1502 supplementation. Avizyme 1502 did not have a significant effect on tissue protein content, activity of the digestive enzymes (except for alkaline phosphatase, of which the activity was significantly ($P<0.05$) reduced due to enzyme addition), nutrient digestibility (protein, gross energy, phosphorus and calcium) and amino acid digestibility in this experiment.

In Experiment 2, three hundred and twenty day-old Ross male chicks were given four main diets. The four main diets were based on a commercial soyabean concentrate and hybrid yellow maize that was sun-dried (control) or oven-dried at temperatures of 85°C,

95°C or 105°C. The oven-dried maize were dried for 24 hours. Each diet was in addition either supplemented with Avizyme 1502 at 1 g/kg diet, or was left without the Avizyme supplement. The birds were given experimental feed from day one to day 28 and the experiment was a completely randomised design with 4x2 factorial arrangement of treatments, and six replicates. Heat treatment of maize significantly ($P<0.05$) improved food intake and feed conversion efficiency. Above a drying temperature of 95°C, heat negatively affected these variables. The digestibility of phosphorus and calcium were also improved by heat treatment of maize. Heat did not have an effect on the activity of the digestive enzymes, tissue protein and amino acid digestibility (threonine, methionine, lysine and phenylalanine). Avizyme 1502 supplementation significantly ($P<0.05$) improved weight gain, sucrase activity and the jejunum protein. Its addition did not have a significant effect on nutrient digestibility (protein, gross energy, phosphorus and calcium), activity of the rest of the digestive enzymes and the digestibility of amino acids.

In Experiment 3, two hundred and forty day-old Ross male chicks were reared separately in this study. Chicks were allocated to battery cages and assigned to one of the dietary treatments. The two main diets were based on a commercial soyabean concentrate and hybrid yellow maize or open pollinated maize respectively. The open pollinated maize was grown in the northern part of KwaZulu-Natal (Hlabisa). Each diet was in addition supplemented with Avizyme 1502 at 1 g/kg diet. The birds were given experimental feed from day one to day 21 and the experiment was a completely randomised design with 2x2x2 factorial arrangement of treatments, and 6 replicates. Maize type used and sex of birds had no significant effect on chicken performance, nutrient digestibility, activity of digestive enzymes and amino acid digestibility (threonine, methionine, lysine and phenylalanine). Although it was not statistically analysed, the chemical composition of the two maize varieties suggested that open pollinated has a more concentrated nutrient and energy content than hybrid maize. This suggests that rural people can be encouraged to continue feeding open pollinated maize to their chickens, and to continue conserving this plant genetic resource.

GENERAL INTRODUCTION

Surveys conducted in South Africa for the periods 1989, 1997, 1998 and 1999 showed that there is a large increase in the production of poultry meat (FAO, 2000). Demand for poultry products, including broiler meat, is also on the increase. The challenges to the industry to meet this demand and sustain the increasing trend in growth are also increasing.

In South Africa, broiler diets are formulated based on cereals. Maize and sorghum are amongst the cereals that are used in formulating these broiler diets. The presence of anti-nutritional factors in most cereals reduces their nutritional value for broilers (Friesen *et al.*, 1992).

Sorghum contains anti-nutritive substances, the most important of which are tannins (Gualtieri and Rapaccini, 1990). For non-ruminants, tannins are known to lower the nutritive value, palatability and protein utilization of the sorghum-based diet (Gualtieri and Rapaccini, 1990). Tannins reduce the digestibility of proteins by forming complexes with them as well as with some digestive enzymes and to a lesser extent with starch. Chickens appear to be affected to a greater extent than pigs (Farrell *et al.*, 1999).

On the other hand, maize is low in non-starch polysaccharides (NSP), the principal anti-nutritive factors that are present in most temperate cereals (Choct and Annison, 1990). Utilization of nutrients contained in maize by broilers is generally considered to be high (Zanella *et al.*, 1999). Although the low NSP content of maize is an advantage, the nutritive value of maize is known to vary widely as a result of climatic conditions during growth and harvest, as well as conditions during post-harvest processing and storage (Barrier-Guillot *et al.*, 1993).

There is no literature showing whether there is a difference in the nutritive value of diets formulated with open pollinated maize or with hybrid maize when fed to broilers. Effects of maize types on the performance of different sexes have not been examined. It is well known that, generally male broilers grow faster than females (Urbasek and Tajovsky, 1991).

Nutritionists have developed different methods of solving the problems caused by anti-nutritional factors in broiler diets. Some of the methods that are used to improve the nutritive value of cereal diets are heating of the cereals, and or addition of the microbial enzymes.

The beneficial effects of some feed enzymes for improving nutrient availability and bird performance are well established. There is also a wealth of data reported on enhancing the feeding value of the coarse cereals by supplementing diets with various enzymes (Bedford, 1996). Enzymes degrade the NSP present in coarse cereals such as wheat, sorghum, barley, rye and oats, thus significantly improving their available energy content. Maize, with a very low NSP level, does not respond to such enzyme treatment. However, in recent years it has been shown that a mixture of enzymes containing amylase, xylanase and protease is effective in enhancing the nutritive value of maize-soyabean meal diets (Bedford, 2000).

Avizyme 1502 (Finnfeeds International, UK) is one of the commercially available enzyme preparations (with a combination of enzymes that target different substrates) that is used to alleviate the problem of anti-nutritional factors in cereals. Its guaranteed minimum activity is 600 U endo-1,4 â-xylanase (EC 3.2.1.8), 8000 U subtilisin (protease, EC 3.4.21.62) and 800 U á-amylase (EC 3.2.1.1) per gram.

The objectives of this research were to: (a) Determine the effect of enzymes on anti-nutritional factors in maize-based diets. (b) Determine the effect of post harvesting treated and enzyme supplementation on maize-based diets. (c) Determine the effect of sex of birds, maize type (open pollinated vs hybrid maize) and Avizyme 1502 supplementation on the nutritive value of maize-based diets for broiler feeding.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

There is a great need for farmers to produce enough food to meet the needs of the burgeoning world population. In this regard, poultry is perhaps more important to human welfare than ever before in history. Poultry products are a dependable source of life-supporting nutrients. Survey of food production and utilization in South Africa for the years 1989, 1997, 1998 and 1999 show that there is a large increase in consumption of poultry meat (FAO, 2000).

Since there is an increase in the demand for poultry meat, there is also a great need to provide broilers with food of high quality to maximize growth and to produce meat of good quality at a reasonable price. The quality of broilers being produced is a reflection of the quality of feed and feeding programs provided by the feed industry (Whitehead, 1991). The formulation of animal diets is quite variable and is dependent upon the cost of ingredients, which in turn is dependent upon their availability. As a result, regional differences in the formulation of the broiler diets can be quite dramatic (Bedford, 1995). The high cost of ingredients in many African countries has caused many poultry farmers to abandon the industry (Onyenokwe, 1994). Feed cost in South Africa represents more than 80% of the total cost of growing broilers (Swatson, 1998). Consequently, in developing countries, the primary energy sources for broiler diets have been the traditional cereals (Friesen *et al.*, 1992). Most of these cereals are of low nutritive value for poultry feeding, since they have anti-nutritional factors (Bedford, 2000).

The main objective of research in this field should therefore be to enable poultry farmers to use a combination of feed ingredients in which relatively large savings in feed costs can be obtained, to avoid further deterioration in profitability. Alternatively, the available ingredients have to be used more efficiently, by making them more palatable and acceptable to the chicken.

1.2 Energy feedstuffs for poultry feeding

Poultry require energy for growth, maintenance of body tissue, production, regulation of body temperature and activity. Like other simple-stomached animals, poultry require feeds that can be digested by enzymes secreted by tissues and associated organs of the alimentary tract. There is a large number of ingredients that can be used as energy feedstuffs. Some of these ingredients are of high quality and others are of low nutritive value. Ingredients of high quality are not readily available to farmers, due to high cost. These energy-containing ingredients are generally low in fibre content, but rich in protein, starch and lipids (Touchburn *et al.*, 1999). Some of these feedstuffs can be grouped into tubers and cereals respectively, as discussed below.

1.2.1 Tubers

Some of the tubers that can be used in poultry feeding are potatoes, cassava and sweet potatoes. Tubers have a comparatively low crude fibre content, and consequently are more suitable than roots for feeding pigs and poultry (Whitehead, 1991). The starch content of the dry matter in tubers is about 700 g/kg; this carbohydrate is present in the form of granules, which vary in size depending upon variety (Touchburn *et al.*, 1999). They differ from the root crops in that they contain either starch or fructan, instead of sucrose, as the main storage carbohydrate.

1.2.2 Cereals

Cereal grains are essentially carbohydrate concentrates, the main component of the dry matter being starch, which is concentrated in the endosperm. The cereal grains that can be used as energy feedstuffs for poultry are barley, oats, wheat, sorghum, maize and rye, in addition to a few localized species. The dry matter content of the grain depends on the harvesting period and storage conditions, but is generally within the range of 800 to 900 g/kg. Cereal starches consist of about 25 % amylose and 75 % amylopectin (Whitehead, 1991).

Despite the favourable nutrient composition of some cereals, their feeding value is particularly poor for poultry, due to the presence of viscous polysaccharides in the endosperm cell wall (Moran *et al.*, 1969; Antoniou and Marquardt, 1981; Ward and Marquardt, 1987; Bengttson and Aman, 1990). The most common anti-nutritional factors, which exert a negative effect on digestion, are the non-starch polysaccharides, mainly the arabinoxylans and ß-glucans.

Earlier studies have identified the soluble ß-glucans and arabinoxylans as being the fractions responsible for impeding digestion through the creation of a viscous intestinal environment (Antoniou and Marquardt, 1982; White *et al.*, 1983). Isolation of these fractions and re-feeding to poultry result in many of the same problems seen when the intact grain is fed, suggesting that NSP are the anti-nutritive factors (White *et al.*, 1981; Antoniou and Marquardt, 1982). Both the ß-glucans and arabinoxylans exert their negative effect on digestion by creating very large entanglements, which results in an elevation of the viscosity of the contents of the small intestine. The increased viscosity of intestinal digesta results in a reduction in the rate of digestion, an elevation of microbial activity in the intestine, a reduction in feed intake and wet litter (Bedford, 1995).

1.3 Maize as an energy source for poultry feeding

The mature maize kernel is made up of three main parts, the seed coat or pericarp, the starchy endosperm and the embryo, which will develop into a new plant upon germination. The endosperm is the main energy reserve of the kernel. In most maize types, this part makes up about four-fifths of the whole kernel by weight. It consists of about 90 % starch and 7 % protein, with small amounts of oils, minerals and other chemical constituents. The average maize kernel contains about 72.8 % carbohydrate, 9.0 % protein and 4.3 % amylose starch (Touchburn *et al.*, 1999). Maize is the most widely used high energy feed for poultry feeding around the world (Bedford, 2000). Maize is low in NSP, the anti-nutritive factor that is present in most temperate cereals (Choct and Annison, 1990). The nutritive value of maize is known to vary widely as a result of climatic conditions (Barrier-Guillot *et al.*, 1993). A study by Leeson *et al.* (1993) revealed that over 25 samples of maize collected from the 1992 Ontario maize harvest yielded a very variable AME, with almost 2.0925 MJ/kg difference between the best and worst samples. These changes reduce the digestibility of starch and availability of amino acids, especially lysine (Barrier-Guillot *et al.*, 1993). Most of the feed companies use hybrid maize to formulate diets for chickens, whereas open pollinated maize is still widely used by rural people for poultry feeding. Little is known about the nutritive value of open pollinated maize for poultry feeding.

1.4 Sorghum as an energy source for poultry feeding

Sorghum is fifth in importance, based on tonnages produced, among the world cereals, after wheat, rice, maize and barley (FAO, 2000). Sorghum, together with millet, is the typical cereal grown in semi-arid areas of the tropics and subtropics. In contrast to industrialized countries, sorghum is particularly important in developing countries, where this grain is mostly utilized as human food (Gualtieri and Rapaccini, 1990). In comparison with maize, sorghum has a similar average content of lysine, methionine, fibre, crude ether extract, ash and phosphorus, but almost twice as much calcium. Nevertheless, sorghum is not considered a very competitive feedstuff, because of its high content of anti-nutritional factors in some varieties (Gualtieri and Rapaccini, 1990). One characteristic of sorghum is its high content of tannins, which are polyphenols found in the testa.

Tannins act as anti-nutritional factors, with widely varying effects, according to their composition and extent of polymerisation. For non-ruminants, tannins are known to lower nutritive value, palatability and protein utilization in sorghum-based diets (Gualtieri and Rapaccini, 1990). In the gastrointestinal tract, tannins are hydrolysed to gallic acid and partially excreted in the form of 4-ortho-methyl gallic acid, utilizing the methionine and choline of the feed as a source of methyl groups for the ortho-methylation (Potter and Fuller, 1968). High concentrations of tannins have been shown to reduce feed intake (Rostagno *et al.*, 1973), nutrient digestibility and N retention (Vohra *et al.*, 1966; Nelson *et al.*, 1975; Elkin *et al.*, 1978). Such effects may explain the growth depression observed in broilers fed on sorghum (Rostagno *et al.*, 1973; Armstrong *et al.*, 1974). Nevertheless, high tannin sorghum cultivars are still widely grown because of their reduced pre-harvest germination (Harris and Burns, 1970), their improved weathering characteristics (Harris and Burns, 1973) and a reduced depredation of grains by birds (Gous *et al.*, 1982).

1.5 Improving the nutritive value of cereals for poultry feeding

Many cereals contain a number of components that cannot be digested by monogastric species because of the lack or insufficiency of endogenous enzyme reaction. In addition to being unavailable to the animal, these components also lower the utilization of other dietary nutrients, leading to depressed animal performance (Ravindran *et al.*, 1999).

In the past, most of the feed companies used antibiotics as feed additives to alleviate the problems caused by anti-nutritive factors in the feed and by microbial populations in the intestine. These antibiotics have been used for many years by the poultry industry and have proved to be an effective way of enhancing animal health, uniformity and production efficiency (Bedford, 2000). Antibiotics function by modifying the intestinal micro-flora by directly killing the micro-organisms (Bedford, 2000). The presence of these intestinal micro-flora is thought to reduce animal efficiency through competing with the host for nutrients in the intestinal tract; through causing diseases, particularly by necrotic enteritis; and through lowering digestive efficiency, by degrading the digestive enzymes; and by reducing the absorptive surface areas. Antibiotic growth promoters exert no benefits on the performance of germ-free animals (Bywater, 1998), an aspect which clearly points to their effect being one of anti-microbial activity, rather than by direct interaction with the physiology of the animal. With a decline in the use of dietary antibiotics, the production efficiency of broilers has been reduced (Bedford, 2000) and feed companies now have to rely more heavily on agronomic practices, feed processing (including pelleting, grinding, heating, etc.), and the use of microbial enzymes to improve the quality of cereals for poultry feeding.

1.5.1 Heat processing

Heating may destroy toxic and anti-nutritional factors in feed ingredients. Heating of cereals causes a reduction of water content in the grain, thus resulting in an improved concentration of nutrients. The starch content of grain can be affected (increased) by heating. These changes in nutrient composition of cereals can have an effect on chickens fed diets based on those cereals. Barrier-Guillot *et al.* (1993) reported that when diets based on sun-dried maize were fed to broiler chickens, there was a reduction in weight gain and feed conversion efficiency.

1.5.2 Microbial enzyme supplementation

The benefits of utilising dietary enzymes are due to the increase in the rate of digestion and availability of sugars. As a result of such improvement in the digestibility, there is a significant change in the substrate quality and the quantity available to the intestinal flora (Bedford, 2000). As digestion improves, the absorption of nutrients will also increase, thus leaving little food for the bacteria, which will cause them to die and decrease in number, giving rise to an improved performance (Petterson and Aman, 1989). This effect is illustrated in Figure 1.1.

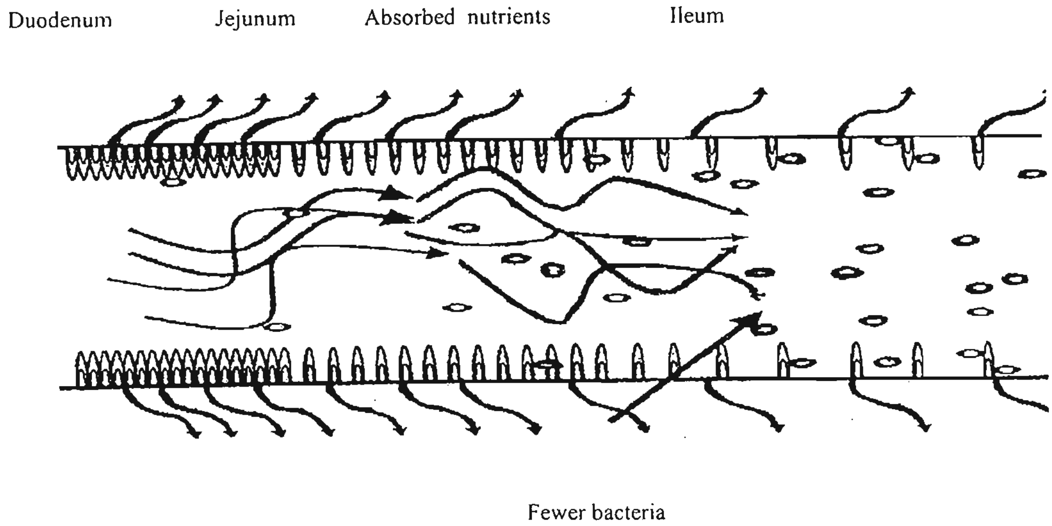

Figure 1.1 Diagrammatic view of the process of digestion under ideal circumstances where the removal of nutrients limits the number of microflora (Bedford, 2000).

Enzymes used in animal feeding are primarily derived from bacterial and fungal fermentation (Bedford, 2000). Commercial products can be crude mixtures of activities or cocktails based on mixtures of specific enzymes. However, specific enzymes are strictly limited in their catalytic capacity and the environmental conditions under which they function. Consequently, successful application of enzyme technology relies on knowledge of the reaction to be affected and the conditions under which the reaction will occur (Bedford, 2000). A major advantage in using enzyme supplementation in poultry diets is that ingredients that are otherwise not used at all or included only at lower levels may be utilized to a greater extent, without loss of performance and at lower cost than conventional ingredients.

A problem associated with enzyme supplementation of monogastric diets is the relatively short transit time of feeds through the gastro-intestinal tract. Thus, apart from the fact that enzymes need to be present in sufficient quantity, they must act rapidly and be resistant to the conditions that exist in the gastro-intestinal tract (Bedford, 2000). The gain achieved through enzyme supplementation of a rye-based poultry diet decline with age of the birds, and this effect is probably due to an increased digestive capacity and gut maturity of older birds (Bedford, 1995). The response to dietary enzyme is substantially greater from poorer quality cereals than is the case with good quality cereals. The enzyme activity is divided into the ileal and caecal phase.

1.5.2.1 Ileal phase

When diets are supplemented with enzymes, there is less undigested starch and protein in the anterior part of the small intestine, which can provide substrate for the endogenous micro-flora. As a result, microbial populations are reduced, and so is the competition for intestinal nutrients (Bedford, 2000). The reduction of micro-flora also reduces the risk of disease outbreak (Bedford, 2000). How much of the response to enzyme utilisation is the result of improved nutrient utilisation and how much to consequential reductions in microfloral population is not known. It is, in fact, impossible to separate the two because the consequence of supplying more substrate to the ileal microflora and thus increasing their populations often results in a subsequent reduction in digestive efficiency from their interaction with the bile salt and digestive enzymes. Recent evidence (Bedford, 1999) suggests that the consequence of a reduced rate of digestion is much more radical in the presence of intestinal micro-flora than in their absence. For example, reducing the rate of digestion of a maize-based diet by inclusion of a viscous pectin or ß-glucan analogue in the diet of a conventional bird, significantly decreases performance (Smits and Annison, 1996; Schutte and Langhout, 1999). When a similar diet was fed to germ-free chickens, no such depression in digestibility or performance was noted.

1.5.2.2 Caecal phase

As the dietary enzyme breaks down viscous ß-glucans and arabinoxylans in the gastro-intestinal tract, they produce small oligomers and free sugars as their end products. Some of these are poorly absorbed by the bird (Schutte, 1990; Verstegen *et al.*, 1997). These sugars provide a fermentation source for specific bacteria and as a result, on entrance into the caeca, they stimulate their growth to varying degrees (Imaiuzumi *et al.*, 1991; Hartemink *et al.*, 1996; Jaskari *et al.*, 1998). Through their activity in providing sugar, enzymes may actually increase the total caecal microbial population. In the caeca, enzymes may contribute to the net energy supply of the bird by encouraging production of the volatile fatty acids, which can be used as an energy source (Bedford, 2000). Table 1.2 shows the results that were obtained by different researchers when enzymes were supplemented to a variety of cereal diets.

Table 1.2 *The effect of enzyme supplementation on the nutritive value of cereals, as measured by chicken performance.*

Reference	Substrate	Enzyme	Enzyme Con(g/kg)	Weight gain (%)[1]	Feed:Gain ratio (%)[1]	AMEn (%)[1]
Friesen *et al.* (1992)	Wheat	ß-glucanase	0.04	+2.9	+9.2	+4.1
	Rye	ß-glucanase	0.04	+29.6	+20.6	+14.0
	Barley	ß-glucanase	0.04	+49.0	+24.2	+42.0
Friesen *et al.* (1991)	Rye*	Pentosanase	0.05	+130	+35.0	-------
	Rye**	Pentosanase	3.2	+193	+43.0	+23.0
Zanella *et al.* (1999)	Maize	Phytase	1	+3.0	-----	-----

[1] Percentage response relative to the unsupplemented control diet.

* Lowest enzyme concentration used.

** Enzyme concentrations corresponding to maximum performance.

1.6 Factors influencing enzyme activity

The optimal enzyme activity in the diet is influenced by the length of the gastro-intestinal tract, pH in the gastro-intestinal tract, dietary pre-treatment, degree of hydration, the body temperature of the animal, as well as the interaction between exogenous and endogenous enzymes. Other factors that influence the optimal enzyme activity are the concentration of products due to enzyme hydrolysis, endogenous enzyme activity/concentration and the type of ingredient (Bedford, 1995).

1.7 Motivation for the study

Maize and sorghum are some of the premier cereals used in poultry feeding around the world. Maize is low in non-starch polysaccharides, the principal antinutritive factors that are present in most temperate cereals (Choct and Annison, 1990). In spite of this advantage, the nutritive value of maize is known to vary widely, as a result of climatic conditions during growth and harvest as well as post-harvest processing and storage (Barrier-Guillot *et al.*, 1993). Maize starch may retrograde or anneal following heating and cooling (Brown 1996; Bedford, 1999). These changes reduce the digestibility of starch and availability of amino acids, notable lysine (Barrier-Guillot *et al.*, 1993).

On the other hand, sorghum contains tannins. For non-ruminants, tannins are known to lower the nutritive value, palatability and protein utilization in sorghum-based diets. It has not been conclusively verified if the reduction in nutritive value can be ameliorated through supplementation of diets with suitable microbial enzymes. Coon *et al.* (unpublished, cited by Bedford, 1999) observed improvements in the ileal but not faecal digestibility of maize starch when maize-targeting enzyme complexes were included in the diet of broiler chickens. Maize obtained from France and South Africa also showed similar variability in response to microbial enzyme supplement (Bedford, 1999). It is also not known whether the nutritive value of open pollinated maize is different from that of hybrid maize for broiler feeding.

Traditionally, maize is still harvested and sun-dried in many parts of Africa. This traditional method is associated with stalk-burn and has been shown to reduce amino acid contents and *in vitro* digestibility, using pancreatin (Panigrahi *et al.*, 1996). It is not known if the nutritive quality of maize could be sustained or enhanced through earlier harvest and artificial drying of the grains. There is also a need to determine the ideal temperature necessary for sustenance of nutritive value.

1.8 Potential benefits of the research

One of the benefits of this research is the determination what level of tannin inclusion broilers will tolerate. The research will also provide information on the relative quality of open pollinated maize, compared to hybrid maize. In addition the effects of artificial drying of maize were evaluated, in order to determine what benefit or otherwise could be derived from this method of processing. In all cases, the effects of a prominent microbial enzyme were evaluated, providing clues for further development by the feed company.

CHAPTER 2

THE EFFECT OF SUPPLEMENTAL TANNIN AND AVIZYME 1502 ON THE NUTRITIVE VALUE OF MAIZE-SOYABEAN BASED DIETS FOR BROILER CHICKENS

2.1 Introduction

A major part of formulated feeds in South Africa is based on cereals. Maize and sorghum are two major cereals that are used for poultry feeding in this country. Maize is low in non-starch polysaccharides (NSP), the anti-nutritive factor present in most temperate cereals (Choct and Annison, 1990). Utilization by broilers of nutrients contained in maize is generally considered to be high (Zanella *et al.*, 1999).

Sorghum is indigenous to arid and semi arid regions of the globe and is one of the most drought-resistant cereal crops. Sorghum is unique among the cereals in that certain cultivars are able to produce relatively large amounts of polymeric, polyphenolic anti-nutritive compounds known as tannins. The adverse effects that result from feeding diets containing high levels of tannin to poultry include reduced growth rates, poorer efficiency of feed utilization and increased incidence of leg abnormalities (Jansman, 1993). In addition, amino acid digestibilities and metabolisable energy content both have been observed to vary inversely with tannin content in the diet, a relationship that has been investigated over the past two decades by several researchers (Kirby *et al.*, 1983; Mitaru *et al.*, 1983; Schang *et al.*, 1991).

The beneficial effects of some feed enzymes for improving nutrient availability and bird performance are well established (Bedford and Morgan, 1996). However there is no record of tackling the tannin problem with enzymes. Avizyme 1502 contains protease, and tannins bind protein, but it has not been demonstrated if enzymes could improve the value of diets containing tannins for broiler feeding.

The objective of the present study is to determine the effect of different tannin levels when supplemented to a maize-based diet, with or without Avizyme 1502, on the nutritive value of the diet and chicken performance. The mechanisms underlying the effects of dietary tannins and the microbial enzyme supplement were also investigated.

2.2 Materials and methods

2.2.1 Experimental animals and feeding

Six hundred day-old Ross male chicks were used for this study. The chicks were randomly allocated to battery cages in groups of 10 and assigned to one of the 5 main diets (Table 2.1). The 5 main diets were based on yellow maize and a commercial soyabean concentrate (Central Soya European Potein A/S, Denmark) and supplemented with tannin (Wattle Bark Industry, S. A. Pty. Ltd, Pietermaritzburg, RSA) at 0, 5, 15, 20 or 25 g/kg feed. In addition each of the diets was supplemented with a microbial enzyme, Avizyme 1502 (Finnfeeds International, UK) at the rate of either 0 g/kg or 1 g/kg. The guaranteed minimum activity of the supplemental enzyme is 600 U endo-1,4 -xylanase (EC 3.2.1.8), 8000 U subtilisin (protease, EC 3.4.21.62) and 800 U -amylase (EC 3.2.1.1) per gram. The experiment was therefore a 5×2 factorial design with 6 replicates per main treatment. Celite, a source of acid-insoluble ash (Poolbrite S.A. Pty. Ltd), was also included in the diets to enable the assessment of nutrient digestibility. The diets were fed for 22 days. Feed and water were supplied *ad libitum*. The room temperature was managed according to the requirements for brooding chicks between hatch and 21 days.

Table 2.1 *Formulated composition (g/kg) and calculated analysis (g/kg) of the diets used in the experiment (as is basis)*

	Tannin (g/kg diet)				
Ingredient	**0**	**5**	**15**	**20**	**25**
Yellow maize	608.90	608.90	608.90	608.90	608.90
Soyabean concentrate	200.00	200.00	200.00	200.00	200.00
Fish meal	60.00	60.00	60.00	60.00	60.00
Sunflower oil	42.70	42.70	42.70	42.70	42.70
Limestone	13.40	13.40	13.40	13.40	13.40
Monocalcium phosphate	12.00	12.00	12.00	12.00	12.00
Celite	30.00	30.00	30.00	30.00	30.00
Salt	2.56	2.56	2.56	2.56	2.56
Plasterer's sand (filler)	25.00	20.00	10.00	5.00	0.00
DL-methionine	1.56	1.56	1.56	1.56	1.56
L-lysine HCl	1.39	1.39	1.39	1.39	1.39
Vitamin/mineral premix	2.50	2.50	2.50	2.50	2.50
Tannin	0.00	5.00	15.00	20.00	25.00
Nutrient composition					
Gross energy (MJ/kg)	16.29	16.60	16.62	16.71	16.97
Crude protein	21.27	21.13	20.62	20.76	20.86
Crude fibre	2.23	1.98	2.99	2.27	2.76
Lipids	6.37	6.87	6.44	6.48	6.45
Calcium	1.15	0.98	1.07	1.13	1.06
Phosphorus (digestible)	0.63	0.60	0.66	0.65	0.65
Methionine	0.46	0.41	0.42	0.43	0.44
Lysine	1.36	1.29	1.33	1.33	1.31

In addition, each diet was supplemented with microbial enzyme, Avizyme 1502 at the rate of 0 or 1 g/kg diet.

2.2.2 Assessment of biological response

The chickens were weighed in groups (chicks in the same cage weighed together) to obtain their body weight at day old and subsequently every 7th day until the end of trial. Feed consumption was recorded at the end of each week. The difference between feed supply and feed leftover in troughs was taken as the feed intake and the feed intake was corrected for mortalities.

2.2.3 Sample collection

At the end of each feeding period (22d or 28d), six chicks per cage were slaughtered by asphyxation with CO_2. Immediately after death, the chicks were dissected to expose the visceral organs. Samples of digesta were collected from the area between Meickel's diverticulum to the ileo-caecal junction. Ileal digesta collected from chickens in each cage, representing one replicate, was pooled and frozen. This sample was subsequently used to determine nutrient digestibility. The pancreas was also removed, weighed and snap-frozen in liquid nitrogen. A 5 cm sub-sample of the jejunum was taken from the proximal end of the region, flushed with ice-cold normal saline (0.9% NaCl) and slit longitudinally. The mucosa was rinsed of digesta with a syringe and the tissue snap-frozen in liquid nitrogen. The jejunal and pancreatic tissues were later processed and used to determine protein content and the activities of digestive enzymes targeting various nutrients.

2.2.4 Preparation of pancreas and jejunum tissues for analysis

The pancreatic tissues were weighed, cut into small pieces and homogenized for one minute using a homogenizer. They were then centrifuged at 23 700 g for ten minutes at 4°C and the supernatant was taken for analysis. The jejunal tissues were prepared according to the method described by Shirazi-Beechey *et al.* (1991). Briefly, the tissue was cut into small pieces and defrosted in a buffer (100 mM mannitol, 2mM *N*-[hydroxyethyl] piperazene- *N'*-[2-ethanesulfonic acid] (HEPES)/ Tris, pH 7.1). About

25 ml of buffer was used for 1 to 2 g of tissue. The mixture was vibromixed for 60 seconds and filtered through a Buchner funnel, 1 mm pore size. The filtrate was blended with a homogenizer for 30 seconds at high speed. One ml of homogenate was then taken for assessment of protein and activities of brush-border membrane-bound digestive enzymes.

2.2.5 Preparation of feed and digesta for analysis

The digesta were freeze dried, weighed and ground in a laboratory mill through a 0.5 mm sieve. The diet was similarly ground through a 0.5 mm sieve and was analysed on an as is basis.

2.2.6 Determination of nutrient and energy concentrations

Protein and amino acids were measured by the method described by Dennison and Gous (1980); and gross energy, phosphorus and calcium were analysed using the AOAC (1990) methods of analysis. Amino acids were analysed on a Beckman amino acid analyser (Beckman Instruments, Inc., Spinco Division, Palo Alto, USA). Calcium was analysed by wet ashing the samples using concentrated sulfuric acid, followed by atomic absorption spectrophotometry. Gross energy was determined with a DDS isothermal CP500 bomb calorimeter (Digital Data Systems (Pty) Ltd., Randburg, Johannesburg, RSA). The AMEn and TMEn were determined by the method of Fisher (1982).

2.2.7 Ileal digestibility of nutrients

Celite, a source of acid insoluble ash (AIA), was added to the diets to enable measurement of ileal digestibilities of nutrients. The concentration of AIA in the diet and digesta was measured as described by Choct and Annison (1992). Diet (2 to 3 g) and digesta (1 g) samples were dried, weighed, ashed (480°C) for 8 h and then treated twice with boiling hydrochloric acid (4 M). The residue was washed, dried (180°C) for 6 h and collected as acid-insoluble ash. The nutrient digestibility was calculated as shown below:

Digestible nutrient =[((nutrient/AIA)$_{diet}$-(nutrient/AIA)$_{ileal\ digesta}$)/(nutrient/AIA)$_{diet}$]

Where: (nutrient/AIA)$_{diet}$ = ratio of nutrient to acid insoluble ash in diet; and (nutrient/AIA)$_{ileal\ digesta}$ = ratio of nutrient to acid insoluble ash in ileal digesta.

2.2.8 Tissue protein content

The protein content of the pancreas and jejunal mucosa was measured as described by Bradford (1976). The assay utilizes the red form of Coomassie Brilliant Blue G-250 (CBB), which turns blue on binding to protein. The reaction was started by adding 2 ml of CBB to 40 μl of dilute homogenate, followed by vibromixing and reading on a UV-Vis spectrophotometer (Hitachi, Japan) at 595 nm after 5 minutes, but within 1 hour. Data generated were analysed with the aid of a computer software programme, Lowry (Elsevier BIOSOFT, Cambridge, UK).

2.2.9 Determination of digestive enzyme activities

2.2.9.1 Alkaline phosphatase (EC. 3.1.3.1)

Alkaline phosphatase was assayed in line with modified methods described by Forstner *et al.* (1968) and Holdsworth (1970). The assay system consists of 50 mM $MgCl_2$, 50 mM Tris (pH 10.1) and the substrate, 10 mM paranitrophenol phosphate (PNP Sigma 104). The standard was paranitrophenol (Sigma 104-1). The reaction was initiated by incubating 25 μl of homogenate with 0.8 ml of 50 mM Tris buffer, pH 10.1; 0.1 ml of 25 mM $MgCl_2$; and 0.1 ml of PNP, at 39°C for 20 minutes. The reaction was terminated with 0.1 ml 40 % trichloroacetic acid (TCA). Further colour development was accomplished by adding 2.0 ml of 0.4 M NaOH to 0.1 ml of the primary reaction mixture, which was then vibromixed and read at 410 nm with an UV-Vis spectrophotometer.

2.2.9.2 Sucrase and maltase

The disaccharidases, -glucosidase (maltase, EC. 3.2.1.20) and -fructofuranodase (sucrase, EC. 3.2.1.26) were assayed using the method described by Dahlqvist (1964). The incubation mixture comprised freshly prepared 100 mM maltose and sucrose, in a succinate buffer (4 mM sodium succinate, 90 mM sodium choride, pH 6.0). Homogenates (25 μl) were incubated in 500 μl of substrate-buffer, for 30 minutes at 39°C. Incubation was terminated by pippeting in five fold of 0.2 % Triton X-100 (w/v) in 0.5 M Tris buffer, pH 7.02 at 39°C. Incubation released glucose, which was then estimated by the GOD-Perid test kit (glucose oxidase, Roche Diagnostics, Indianapolis USA). The amount of glucose released was measured colorimetrically at 610 nm after 30 minutes of colour development at room temperature.

2.2.9.3 Amylase (EC. 3.2.1.1)

The substrate was 2 mg/ml soluble starch (Difco) in 50 mM phosphate buffer (pH 7.2). The original method used a reagent, dinitrosalicylic acid (DNS), made from several chemicals and large volumes of this were required for each assay. The modification retained the first step of Miller *et al.* (1960), in which the substrate (starch) was digested to disaccharides and relatively small amounts of glucose. The latter can be more rapidly measured using the Glucose kit supplied by Roche Diagnostics. In 1.5 ml reaction mixture, 0.5 ml dilute sample and 0.5 ml 50 mM phosphate buffer, pH 7.2. were incubated with 500 μl (1mg starch) pre-warmed substrate for one hour at 39°C. The reaction was terminated with 0.2 % Triton X-100/0.5 M Tris buffer and the glucose formed was measured as described in section 2.7.2.

2.2.9.4 Pancreatic lipase (EC. 3.1.1.3)

The substrate was prepared by suspending 0.5% tributyrin (TB, Kodak) in 100 mM Tris-25 mM $CaCl_2$ buffer (pH 8.0). The stock standard was prepared by dissolving a standard microbial lipase in 50 mM Tris/7.5 mM $CaCl_2$, pH 8.0, to yield 0.5 U/μl. The desired concentration (0.05 U/μl) was obtained by diluting 1 in 10, using the buffer. About 100 μl sample was incubated in 2 ml of pre-warmed (39°C) substrate for 30 minutes, and the reaction was terminated with 100 μl of 40 % TCA. The absorbance was then read at 450 nm (Smeltzer *et al.*, 1992).

2.2.9.5 Chymotrypsin (EC. 3.4.21.1)

The substrate was 0.1 mM solution of succinyl-$(ALA)_2$-Pro-Phe-*p*-nitroanilide (SAPNA) in 50 mM Tris-HCl/20 mM $CaCl_2$ buffer (pH 7.5). For the standard, graded concentrations of *p*-nitroaniline were used. About 30 µl of sample were added to 1.5 ml fresh substrate solution and incubated at 39°C for 10 minutes; the reaction was terminated with 250 µl of 30% acetic acid and the absorbance was read at 410 nm against a water blank (Zaragoza *et al.*, 1997).

2.2.10 Statistical analysis

The data were analysed for significant differences using the General Linear Model procedure (Minitab, 1998). Fisher's pairwise comparison and the t-test were used to compare the treatment means.

2.3 Results

2.3.1 Biological response

There was a significant ($P<0.001$) effect of tannin on weight gain, feed intake and feed conversion efficiency (Table 2.2). There was a negative relationship between tannin content in the diet and feed intake by birds. As the level of tannin increased in the diet, the feed intake by birds decreased ($P<0.001$). There was also a negative relationship between tannin level in the diet and weight gain. The feed conversion efficiency was low at low tannin level and it increased ($P<0.001$) with increasing tannin level up to a point and then dropped with further increase in tannin content of the diet. Avizyme 1502 supplementation had no significant effect on feed intake and feed conversion efficiency by birds. Although the effect of Avizyme 1502 was not significant on feed intake and feed conversion efficiency, there was a slight increase in the feed intake of those birds assigned to diets supplemented with Avizyme 1502 when compared to those given unsupplemented diets. There was no difference in the feed conversion efficiency of birds at low and high tannin levels, but at intermediate level of tannin there was an insignificant increase in the feed conversion efficiency of birds assigned to the diet supplemented with Avizyme 1502 when compared to those given the unsupplemented diet. Avizyme 1502 supplementation significantly (P<0.05) increased body weight gain. The interaction of tannin and Avizyme 1502 did not have any significant effect on feed intake, weight gain and feed conversion efficiency and as a result was not included in the table (Table 2.2).

Table 2.2 *Feed intake (FI), weight gain and feed conversion efficiency (FCE) of the birds given test diets with or without Avizyme 1502*

Tannins g/kg	**FI (g/bird d)**	**Weight gain (g/bird d)**	**FCE (g gain/kg feed intake)**
0	43.94	20.18	460.41
5	38.82	20.32	524.20
15	36.58	17.96	491.42
20	35.57	17.35	488.61
25	35.51	16.5	465.40
s.e.d	20.16	13.38	15.73
Enzyme			
(-) Aviz	37.76	18.02	477.6
(+) Aviz	38.40	18.92	494.40
s.e.d	12.75	8.46	9.95
S.O.V	**P**	**P**	**P**
Tannin	0.001	0.001	0.001
Enzyme	0.276	0.024	0.098

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

2.3.2 Tissue protein concentration and activities of digestive enzymes

There was no significant effect of tannin on the activities of amylase, chymotrypsin and lipase (Table 2.3). Pancreatic tissue protein was significantly (P<0.05) affected by tannin. Avizyme 1502 also had no significant effect on these variables. There was no significant effect of tannin in the activities of maltase, sucrase and alkaline phosphatase (Table 2.4). There was a significant (P<0.05) increase in the tissue protein content of jejunal mucosa with increasing level of tannin in the diet, but at tannin level above 20 g/kg it decreased. Avizyme 1502 supplementation also significantly ($P<0.01$) increased jejunal protein content, but significantly ($P<0.05$) decreased the activities of maltase and alkaline phosphatase. Avizyme 1502 had no effect on the activity of sucrase in the jejumum. The interaction of tannin and enzyme did not have a significant effect on these variables and as a result was not included in the table.

Table 2.3 *The effect of tannin level and Avizyme 1502 supplementation on the pancreatic tissue protein content and activities of pancreatic digestive enzymes*

Tannins g/kg	Protein[1]	Amylase[2]	Chymotrypsin[3]	Lipase[4]
0	87.20	2.48	0.77	0.03
5	101.10	2.62	0.71	0.03
15	96.60	2.79	0.75	0.03
20	97.30	2.91	0.71	0.02
25	96.00	2.63	0.74	0.02
s.e.d	4.50	0.31	0.06	0.002
Enzyme				
(-) Aviz	94.10	2.67	0.72	0.03
(+) Aviz	97.10	2.70	0.75	0.02
s.e.d	2.85	0.19	0.04	0.001
S.O.V	**P**	**P**	**P**	**P**
Tannin	0.048	0.69	0.85	0.822
Enzyme	0.294	0.88	0.34	0.164

[1] = mg/g tissue; [2] = nmole glucose/mg protein/minute; [3] = µmole nitrioaniline/mg µmole nianiline/ mg protein/minute; [4] = lipase unit

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

Table 2.4 *The effect of tannin level and Avizyme 1502 supplementation on the jejunal tissue protein content and activities of intestinal digestive enzymes*

Tannins g/kg	Protein[5]	Maltase[6]	Sucrase[6]	AP[7]
0	55.70	2.03	0.02	1.10
5	48.20	2.15	0.03	1.28
15	63.20	2.18	0.03	1.17
20	70.20	1.99	0.03	1.04
25	63.40	2.07	0.03	1.05
s.e.d	3.34	0.24	0.003	0.19
Enzyme				
(-) Aviz	54.10	2.25	0.03	1 0.29
(+) Aviz	66.20	1.92	0.09	0.95
s.e.d	4.01	0.15	0.002	0.12
S.O.V	**P**	**P**	**P**	**P**
Tannin	0.013	0.935	0.141	0.699
Enzyme	0.004	0.034	0.487	0.006

[5] =mg/g tissue; [6] = µmole glucose/mg protein/minute; [7] = µmole PNP/mg protein/minute.
AP = Alkaline phosphatase
SOV =Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

2.3.3 Ileal digestibility of nutrients

There was a significant (P<0.000) negative relationship between dietary tannin level and ileal digestibility of protein, gross energy (P<0.000) and phosphorus (P<0.05) (Table 2.5). At the low levels of tannin inclusion, the digestibility of these nutrients rose with increasing level of tannin up to a point and then started to decrease with further increase in tannin. Tannin inclusion in the diet had no significant effect on calcium digestibility. Avizyme 1502 also had no significant effect on the digestibility of protein, gross energy, phosphorus and calcium. The interaction of tannin and Avizyme 1502 had no significant effect on the digestibility of these nutrients and was not includes in the table. The inclusion of 5, 15, and 20 g tannin per kg diet significantly (P<0.001) reduced threonine digestibility (Table 2.6). When tannin inclusion in the diet was increased to 25 g/kg diet, threonine digestibility was significantly increased. The digestibility of methionine and phenylalanine were significantly (P<0.01) reduced with increasing level of tannin in the diet. Inclusion of tannin in the diet did not influence the digestibility of lysine. There were no significant effects of Avizyme 1502 on the ileal digestibility of the amino acids mentioned above.

Table 2.5 *The effect of tannin level and Avizyme 1502 supplementation on the ileal digestibility of nutrients (expressed as proportions).*

Tannins g/kg	Protein	Gross energy	Phosphorus	Calcium
0	0.79	0.77	0.45	0.58
5	0.80	0.78	0.49	0.49
15	0.78	0.79	0.59	0.59
20	0.76	0.77	0.57	0.57
25	0.73	0.69	0.50	0.53
s.e.d	0.01	0.03	0.04	0.05
Enzyme				
(-) Aviz	0.77	0.75	0.53	0.57
(+)Aviz	0.77	0.77	0.52	0.53
s.e.d	0.01	0.02	0.02	0.03
S.O.V	**P**	**P**	**P**	**P**
Tannin	0.001	0.071	0.053	0.190
Enzyme	0.712	0.270	0.690	0.211

SOV =Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

Table 2.6 *The effect of tannin level and Avizyme 1502 supplementation on the amino acid digestibility (expressed as proportions)*

Tannins g/kg	Lysine	Methionine	Phenilinine	Threonine
0	0.89	0.92	0.86	0.71
5	0.89	0.91	0.87	0.66
15	0.88	0.88	0.84	0.63
20	0.87	0.86	0.83	0.61
25	0.86	0.85	0.79	0.96
s.e.d	0.01	0.02	0.02	0.03
Enzyme				
(-) Aviz	0.88	0.89	0.84	0.72
(+) Aviz	0.87	0.88	0.84	0.70
s.e.d	0.01	0.01	0.01	0.03
S.O.V	**P**	**P**	**P**	**P**
Tannin	0.09	0.002	0.003	0.001
Enzyme	0.450	0.395	0.700	0.449

SOV =Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = P-probability

2.4 Discussion

2.4.1 Biological response

The present study demonstrated that the performance of broiler chickens was negatively affected by the presence of tannin in the diet. Feed intake, weight gain and feed conversion efficiency were significantly reduced as the tannin level in the diet increased. These results are in agreement with the results by Gualtieri and Rapaccini (1990) who reported that for non-ruminants, tannins are known to lower the nutritive value, palatability and protein utilization of the sorghum-based diet. The feed conversion efficiencies in this experiment are far below that of the expected FCE for Ross broilers at three weeks of age under commercial conditions, which is 770 g gain/kg food intake (Ross, 1999). This may be attributed to the fact that these birds were give experimental diets, which consisted of ingredients such as sand (0 to 2.5 % inclusion) and celite (3 % inclusion) which are non-digestible ingredients. Farrell *et al.* (1999) reported that tannin reduces the digestibility of protein by forming a complex with them as well as with some digestive enzymes, and to a lesser extent starch, thus decreasing chicken performance. This response may be dependent on the concentration of tannins in the diet (Nyachoti *et al.*, 1996; Pour-Reza and Edriss, 1997). In the present study, lower levels of tannin were less detrimental to digestibility and productivity than higher levels. It would appear that poultry tolerate tannins level below 5 g/kg, but the exact mechanisms are not well understood.

Avizyme 1502 supplementation in the diet significantly improved ($P<0.05$) the weight gain. The improvement in the weight gain of broiler chickens given maize-based diets supplemented with Avizyme 1502, as observed in the present study, is in agreement with the results reported by previous researchers (Petterson *et al.*, 1990; Bedford and Classen, 1992; Friesen *et al.*, 1992; Veldman and Vahl, 1994; Choct *et al.*, 1995). There were no major effects of the microbial enzyme on feed intake and utilization (FCE). This effect is in contrast to the effect of the supplement on wheat-based diets (Steenfeldt *et al.*, 1998) and may be due to differences in levels of NSP in the two cereals. There is no work that has been previously done on the effect of Avizyme 1502 on the nutritive value of diets containing tannins for broiler feeding. Since Avizyme 1502 contains protease, and tannin binds protein, the positive effect of Avizyme 1502 supplementation on chicken performance in this experiment can be attributed to protease breaking down the binding effect of tannins on protein, thus making protein available for digestion and absorption. This results in an improvement in chicken performance. Ingredients high in tannin content should not be used for broiler feeding since they decrease chicken performance.

2.4.2 Ileal digestibility of nutrients

The results of the present experiment shows that increasing the inclusion of tannin from 0 g/kg to 5 g/kg improved the digestibility of protein, gross energy and phosphorus. When the inclusion of tannin was above 5 g/kg, the digestibility of protein, gross energy and phosphorus was significantly reduced. This is attributable to the binding effect of tannin on nutrients, which causes dietary nutrients to be unavailable for digestion in the digestive tract of the bird (Gualtieri and Rapaccini, 1990). The results of this experiment are similar to the report by Flores *et al.* (1994) that addition of tannin extract in the diet decreased the digestibility of starches. No work has been done on improving the nutritive digestibility of diets containing tannins for broiler by feeding Avizyme 1502. In the present experiment the addition of 1g/kg Avizyme 1502 to the diets did not improve the digestibility of protein, gross energy, phosphorus and calcium. The expectation in this experiment was that Avizyme 1502 would increase the digestibility of protein and other nutrients, since it contains protease which targets proteins. In agreement with previous work (Rostagno *et al.*, 1973; Mitaru *et al.*, 1985 and Schang *et al.*, 1991), results of the present study show that the digestibility of threonine, methionine and phenylalanine is inversely related to the tannin content in the diet. In previous experiments with broiler chicks, Nelson *et al.* (1975) and Kirby *et al.* (1983) reported significant negative correlations between sorghum tannin content and amino acid digestibility. This is attributable to the binding effect of tannin on nutrients, thus making them unavailable for digestion. The present study was unable to detect significant differences in the amino acid digestibility due to Avizyme 1502 supplementation. The reasons for this are not known, and there is a need to test a wider range of enzyme supplementation levels.

2.4.3 Tissue protein content

Tissue protein content is one of the measures of organ/tissue development (Waterlow *et al.*, 1978). In broiler chickens, organs associated with nutrient derivation are preferentially developed early in life (Iji *et al.*, 2001a). It has not been largely established how this process is influenced by the presence of anti-nutritive factors. The results obtained in the present study indicated that tannins influence tissue protein synthesis or accumulation. The effects of the tannin may be due to factors other than gross structural development in the gastrointestinal tract.

2.4.4 Activities of digestive enzymes

2.4.4.1 Jejunal enzymes

There was no significant effect of tannin on the activity of alkaline phosphetase, maltase and sucrase. This result is in contrast to the findings of Majumdar and Moudgal (1994) who obtained an increase in the activity of AP and sucrase when hens were given a single dose of pure tannic acid, at 200 and 300 mg/head daily. This variation may, however, be due to age differences and the level of tannin used. In broiler chickens, the activity of AP has been observed to decline with age between hatch and 21 days (Iji *et al.*, 2001b) but its response to tannins within this age category has not been previously investigated. The reasons for these effects are not fully understood. The activities of endogenous enzymes are dependent on substrate availability. Avizyme supplementation significantly reduced the activity of maltase and sucrase. Exogenous enzyme supplements may reduce the availability of substrates for endogenous enzymes. However, the patterns of activities of both sources of enzymes in broiler chickens have not been previously investigated.

2.4.4.2 Pancreatic enzymes

Tannin inclusion in the diet did not have a significant effect on the activities of amylase, chymotrypsin and lipase. In rats tannins are known to directly interfere with mucosal proteins, thereby stimulating cell renewal (Vallet *et al.*, 1994). If this is also the case with broilers, tannin inclusion may increase the activity of these enzymes, since there would be more cells to secrete the enzymes, but this would depend on the relationship between cell formation and turnover. There are no reports on the effect of Avizyme 1502 on the activities of these enzymes. Poultry saliva is deficient in amylase (Low and Zebrowska, 1986), so pancreatic and intestinal carbohydrases play a significant role in the digestion of ingested sugars. Since Avizyme 1502 contains amylase, the digestion of ingested sugar should have been increased in this experiment, thus decreasing amylase activity in the pancreas.

2.5 Conclusions

There was a negative relationship between dietary tannin and chicken performance. Nutrient digestibility was also negatively affected by tannin. Avizyme 1502 increases chicken performance and digestibility of nutrients. In conclusion, ingredients high in tannin concentration should not be used for broiler feeding.

CHAPTER 3

EFFECT OF MAIZE DRYING TEMPERATURE AND AVIZYME 1502 SUPPLEMENTATION ON THE NUTRITIVE VALUE OF MAIZE-SOYABEAN BASED DIETS FOR BROILER FEEDING

3.1 Introduction

Maize grain is one of the premier cereals used in poultry feeding by commercial feed companies. Maize is low in non-starch polysaccharides, which is an anti-nutritive factor (Choct and Annison, 1990). In spite of this advantage, the nutritive value of maize is known to vary widely, as a result of climatic conditions during growth and harvest as well as post-harvest processing and storage (Barrier-Guillot *et al.*, 1993; Leeson *et al.*, 1993).

In many African countries, maize is sun-dried after harvesting. Heating and cooling of maize grain may cause its starch to retrograde or anneal. These changes reduce the digestibility of starch and availability of amino acids (Barrier-Guillot *et al.*, 1993). When diets based on sun-dried maize were fed to broiler chickens, there is a reduction in weight gain and feed conversion efficiency (Panigrahi *et al.*, 1996). It is not known if the nutritive value of maize could be better sustained or enhanced through artificial drying of maize grains. There is also a need to determine the ideal drying temperature necessary to optimize the nutritive value. Artificial drying is used in temperate countries and sometimes in tropical countries when maize is harvested during wet weather.

It has not been conclusively verified if the reduction in nutritive value of maize due to heating effects can be ameliorated through supplementation of diets with suitable microbial enzymes. Bedford (2000) observed improvement in chicken performance and ileal digestibility of maize starch when maize-based diets were supplemented with Avizyme 1502. The objective of this experiment was to examine the effects of drying temperature and Avizyme 1502 supplementation on the nutritive value of maize-based diets.

3.2 Materials and methods

3.2.1 Experimental animals and feeding

Four hundred and twenty day-old Ross male chicks were used in the experiment. The chicks were randomly allocated to litter cages in groups of 10 and assigned to one of four main diets (Table 3.1). The four main diets were based on soybean isolate and hybrid yellow maize that was either sun-dried (control) or oven-dried (for 24 h) at temperatures of 85°C, 95°C or 105°C (Table 3.2). Each of the four main diets was in addition supplemented with microbial enzyme, Avizyme 1502 (Finnfeeds International, UK) at the rate of either 0 g/kg or 1 g/kg. The experiment was a 4x2 factorial design, with 6 replicates. The guaranteed minimum activity of the supplemental enzyme is described in section 3.2.1. Celite, a source of acid-insoluble ash (Poolbrite S.A. Pty. Ltd), was also included in the diets to enable the assessment of nutrient digestibility. The diets were fed for 28 days. Food and water were supplied *ad libitum*. The room temperature was managed according to the requirements for broiler chicks between hatch and 28 days.

Table 3.1 *Composition (%) and calculated analysis of the basal diets used in the experiment (as is basis)*

Ingredient	Sun-dried diet	85°C diet	95°C diet	105°C diet
Yellow maize	60.89	60.89	60.89	60.80
Soyabean isolate	20.00	20.00	20.00	20.00
Fishmeal 65	6.00	6.00	6.00	6.00
Sunflower oil	4.27	4.27	4.27	4.27
L-lysine HCl	0.14	0.14	0.14	0.14
DL-methionine	0.16	0.16	0.16	0.16
Vitamin/mineral premix	0.25	0.25	0.25	0.25
Limestone	1.34	1.34	1.34	1.34
Monocalcium phosphate	1.20	1.20	1.20	1.20
Salt	0.26	0.26	0.26	0.26
Celite	3.00	3.00	3.00	3.00
Plasterer's sand (filler)	2.50	2.50	2.50	2.50
Nutrient composition				
Gross energy (MJ/kg)	16.30	16.56	16.81	16.75
Crude protein	21.27	21.30	20.42	21.51
Crude fibre	2.23	2.22	2.51	2.69
Lipids	6.37	6.53	6.68	7.01
Calcium	1.15	1.06	1.11	1.00
Phosphorus (digestible)	0.63	0.65	0.63	0.63
Methionine	0.46	0.43	0.41	0.45
Lysine	1.36	1.39	1.30	1.34

In addition, each diet was supplemented with microbial enzyme, Avizyme 1502 at the rate of 0 or 1 g/kg diet.

Table 3.2 *Composition of naturally dried maize and maize dried at different temperatures (g/kg as fed)*

	Drying Temperature of maize			
Nutrient fraction	**Sun-dried**	**85 C**	**95 C**	**105 C**
Dry matter	887.00	915.00	925.00	929.00
Fat	30.90	39.70	37.60	38.10
Protein	69.10	73.10	74.30	75.30
Crude fibre	24.40	20.90	19.10	18.30
GE MJ/kg	16.40	17.00	17.00	16.90
AMEn MJ/kg	13.90	14.60	14.50	14.50
TMEn MJ/kg	14.30	15.00	14.90	14.90
Calcium	0.06	0.06	0.09	0.05
Phosphorus	1.70	2.00	1.90	1.80
Magnesium	0.94	1.06	1.04	1.00
Threonine	24.2	25.5	27.7	26.3
Methionine	21.2	17.1	13.8	15.1
Lysine	21.1	21.4	24.0	21.7
Phenylalanine	31.2	32.1	3.38	34.8

3.2.2 Assessment of biological response

The chickens were weighed in groups (chicks in the same cage weighed together) to obtain their body weight at day old and subsequently every 7th day until the end of trial. Feed consumption was recorded at the end of each week. The difference between feed supply and feed leftover in troughs was taken as the feed intake and the feed intake was corrected for mortalities.

3.2.3 Tissue and digesta collection

Tissue and digesta were collected as described in Chapter 2.

3.2.4 Preparation of tissue, feed and digesta for analysis

Tissue, feed and digesta were prepared as described in Chapter 2.

3.2.5 Determination of nutrients and ileal digestibility of nutrients

Dietary nutrients and ileal digestibility of nutrients were determined as described in Chapter 2.

3.2.6 Determination of tissue protein and the activity of digestive enzymes

Tissue protein and the activities of the digestive enzymes were determined as described in Chapter 2.

3.3 Results

3.3.1 Maize quality

The dry matter, phenylalanine and protein contents of the maize grain tended to increase with increasing drying temperature. Samples were analysed in duplicate, data were not analysed statistically. The crude fibre, GE, AME, TMEn, phosphorus and magnesium contents, increased when the maize was dried at 85°C (Table 3.2). Above 85°C, drying of maize resulted in a reduction of these nutrients. Calcium, threonine and lysine contents increased with increasing drying temperature up to 95°C but a further increase in drying temperature resulted in the reduction of these nutrients.

3.3.2 Biological response

Feed intake and feed conversion efficiency (FCE) were significantly ($P<0.05$) increased as the drying temperature increased up to 95°C but a further increase in the drying temperature resulted in a reduction in feed intake (Table 3.3). FCE was significantly ($P<0.05$) reduced when the maize was dried at 85°C but a further increase in the drying temperature resulted in a decrease in FCE. Drying temperature had no significant effect on weight gain. Avizyme 1502 supplementation had no significant effect on feed intake and feed conversion efficiency. Avizyme 1502 significantly ($P<0.05$) increased body weight gain except for the birds given the diet containing the maize dried at 105°C

Table 3.3 *The effect of drying temperature (Temp) and Avizyme 1502 supplementation on feed intake (FI), weight gain and feed conversion efficiency* (FCE)

Temp	FI (g/bird d)	Weight gain (g/bird d)	FCE (g gain/kg FI)
Sun dried	54.12	27.53	513.10
85°C	57.57	24.92	433.42
95°C	60.11	26.55	442.31
105°C	58.50	26.06	446.80
s.e.d	1.91	0.96	17.34
Enzyme			
(-) Aviz	56.46	25.54	453.50
(+) Aviz	58.5	26.99	464.30
s.e.d	1.35	0.68	12.26
S.O.V	**P**	**P**	**P**
Temp	0.020	0.072	0.001
Enzyme	0.141	0.041	0.380

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

3.3.3 Tissue protein concentration and activities of digestive enzymes

There was no significant effects of drying temperature on pancreatic protein content and on the activities of amylase, chymotrypsin and lipase (Table 3.4). Avizyme and the interaction of drying temperature and Avizyme did not have a significant effect on the activities of these enzymes. Drying temperature had no significant effect on jejunal protein content nor the activities of maltase, sucrase and alkaline phosphatase (Table 3.5). There was a significant increase in the jejunal protein content and in the activity of sucrase ($P<0.01$) due to Avizyme 1502 supplementation. The interaction of drying temperature and Avizyme 1502 supplementation did not have any significant effect on the activity of the digestive enzymes and protein tissues studied in this experiment.

Table 3.4 *The effect of drying temperature (Temp) and Avizyme 1502 supplementation on the pancreatic protein tissue and the activity of the pancreatic digestive enzymes*

Temp	Protein[1]	Amylase[2]	Chymotrypsin[3]	Lipase[4]
Sun dried	59.39	9.60	0.49	0.02
85°C	59.99	10.67	0.50	0.03
95°C	61.87	10.10	0.51	0.03
105°C	57.27	10.66	0.55	0.02
s.e.d	2.670	1.10	0.06	0.003
Enzyme				
(-) Aviz	60.88	10.47	0.54	0.03
(+) Aviz	58.39	10.04	0.49	0.03
s.e.d	1.89	0.78	0.05	0.002
S.O.V	**P**	**P**	**P**	**P**
Temp	0.406	0.73	0.846	0.366
Enzyme	0.199	0.59	0.273	0.777

[1] = mg/g tissue; [2] = nmole glucose/mg protein/minute; [3] = µmole nitrioaniline/mg µmole nianiline/ mg protein/minute; [4] = lipase unit

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

Table 3.5 *The effect of drying temperature (Temp) and Avizyme 1502 supplementation on the jejunum protein tissue and the activity of the digestive enzymes in the jejunum*

Temp	Protein[5]	Maltase[6]	Sucrase[6]	AP[7]
Sun dried	50.60	2.09	0.02	1.33
85°C	45.00	2.72	0.02	1.71
95°C	52.10	2.37	0.03	1.70
105°C	49.60	2.65	0.03	1.62
s.e.d	5.60	0.27	0.004	0.20
Enzyme				
(-) Aviz	45.50	2.50	0.02	1.67
(+) Aviz	53.20	2.42	0.03	1.51
s.e.d	3.96	0.19	0.003	0.14
S.O.V	P	P	P	P
Temp	0.624	0.104	0.257	0.223
Enzyme	0.061	0.666	0.004	0.249

[5]=mg/g tissue; [6]= µmole glucose/mg protein/minute; [7]= µmole PNP/mg protein/minute.
AP = Alkaline phosphetase
SOV =Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

3.3.4 Ileal digestibility of nutrients

When the maize was heated at 85°C and 95°C, the phosphorus digestibility was significantly increased ($P<0.05$). At 105°C, phosphorus digestibility was significantly reduces (Table 3.6). As the drying temperature increased up to 95°C, the digestibility of calcium significantly ($P<0.05$) increased, but a further increase in drying temperature resulted in a decrease in calcium digestibility. Drying temperature did not have an effect on the digestibilities of protein and gross energy. Drying temperature, Avizyme 1502 supplementation and the interaction between Avizyme 1502 and drying temperature did not have a significant effect on the digestibility of threonine, methionine, lysine and phenylalanine (Table 3.7).

Table 3.6 *The effect of drying temperature (Temp) and Avizyme 1502 supplementation on the ileal digestibility of nutrients (expressed as proportions)*

Temp	GE	Protein	Phosphorus	Calcium
Sun dried	0.69	0.77	0.34	0.43
85°C	0.62	0.75	0.50	0.60
95°C	0.63	0.76	0.50	0.66
105°C	0.57	0.73	0.41	0.60
s.e.d	0.04	0.03	0.05	0.04
Enzyme				
(-) Aviz	0.61	0.75	0.44	0.55
(+) Aviz	0.65	0.76	0.44	0.57
s.e.d	0.03	0.02	0.04	0.03
S.O.V	**P**	**P**	**P**	**P**
Temp	0.052	0.506	0.020	0.013
Enzyme	0.251	0.308	0.872	0.582

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

Table 3.7 *The effect of drying temperature (Temp) and Avizyme 1502 supplementation on the ileal digestibility of amino acids (expressed as proportions)*

Temp	Threonine	Methionine	Lysine	Phenylalanine
Sun dried	0.69	0.86	0.84	0.84
85°C	0.66	0.85	0.85	0.84
95°C	0.66	0.87	0.86	0.84
105°C	0.65	0.86	0.84	0.82
s.e.d	0.05	0.03	0.02	0.02
Enzyme				
(-) Aviz	0.65	0.086	0.84	0.82
(+) Aviz	0.68	0.86	0.85	0.84
s.e.d	0.04	0.02	0.01	0.016
S.O.V	P	P	P	P
Temp	0.920	0.924	0.850	0.764
Enzyme	0.391	0.770	0.332	0.373

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance

3.4 Discussion

3.4.1 Maize quality

Although it was not statistically analysed, the content of nutrients in maize tended to increase with increasing drying temperature. When water is removed from the grains through heating, nutrients become more concentrated. Deviations from this expectation, as observed for some nutrients at the highest drying temperatures, may be due to biological variation in the sampled materials and to sampling variation. Drying may also improve the storage time of the grains.

3.4.2 Biological response

The present study demonstrated that feed intake and feed conversion efficiency were significantly improved as the drying temperature increased. However above 95°C they were negatively affected by the increase in drying temperature. The improvement in performance might have come as a result of the improvement in starch and protein digestibility due to the drying process. The decrease in chicken performance at temperatures above 95°C may be attributed to the denaturation of nutrients at high temperatures. Although feed intake and feed conversion efficiency were not significantly affected by Avizyme 1502, there was a tendency for these variables to improve due to Avizyme 1502 supplementation. The poor FCE observed in this experiment, vis-a-viz commercial expectations (as per Ross broiler production manual-Ross Poultry Breeder, 1999) may have been due to the indigestible sand and celite included in the experimental diets.

3.4.3 Ileal nutrient digestibility

The results of the present experiment demonstrate that drying temperature significantly improved phosphorus and calcium digestibility up until 95°C. A further increase in drying temperature negatively affected digestibility. Although maize is different from canola, the results of the present experiment are in agreement with the report by Anderson-Hafermann *et al.* (1993) who reported an improvement in the digestibility of phosphorus, calcium and amino acids when a canola-based diet was heated. In this thesis, the improvement in the digestibility of these nutrients was due to the effect of heat on maize protein and starch. The drying of maize did not significantly affect the digestibility of amino acids (threonine, methionine, lysine and phenylalanine) in this experiment. Avizyme 1502 had no significant effect on the digestibility of the above-mentioned amino acids. We had expected Avizyme to improve digestion of amino acids, since it break down the binding effect of anti-nutritional factors on nutrients.

3.4.4 Activities of digestive enzymes

3.4.4.1 Jejunal enzymes

The present study did not reveal any effects of maize drying temperature on the activities of AP, sucrase and maltase. There are no previous research results to confirm these findings. Dry heat may affect grain structure (Brown, 1996; Bedford, 1996), but digestive enzyme activities do not appear to vary with maize quality observed in this study. The results of this experiment demonstrate that Avizyme 1502 did not have a significant effect on the activity of AP and maltase but did significantly improved the activity of sucrase. These results on the activity of these enzymes are complicated and further research need to be done to determine the effect of Avizyme 1502 and drying temperature on the activities if these digestive enzymes.

3.4.4.2 Pancreatic enzymes

The results of the present study demonstrate no effect of drying temperature on the activities of the pancreatic enzymes (amylase, chymotrypsin and lipase). This may be attributed to the fact that pancreatic enzymes do not respond directly to substrate (Nir *et al.*, 1993), so differences in drying temperature would not affect their activities. Wang *et al.* (1998) reported that the proportional activity of digestive enzymes secreted by the pancreas of sheep varies regardless of the proportion of the substrate in digesta. Avizyme 1502 supplementation did not have a significant effect on the activities of amylase, chymotrypsin and lipase. Dry heat may affect grain structure, but digestive enzyme activities do not appear to vary with maize quality.

3.5 Conclusions

Drying up to a temperature of 95°C improved the nutritive value of maize for broilers. When maize is dried, water is removed and nutrients become concentrated. This increases the chicken performance. Activity of the digestive enzymes seems not to be affected by drying of maize. Farmers should continue drying their maize to increase the nutritive value for broilers, but care should be taken not to dry at temperatures above 95°C. Microbial enzymes are more effective on diets with anti-nutritive factors. Since maize is low in NSP, Avizyme 1502 did not have a significant effect on most of the variables measured in this experiment.

CHAPTER 4

THE EFFECT OF MAIZE GENOTYPE, SEX OF BIRDS AND AVIZYME 1502 SUPPLEMENTATION ON THE NUTRITIVE VALUE OF MAIZE-SOYABEAN BASED DIETS FOR BROILER FEEDING

4.1 Introduction

Variation in cereal composition caused by genetic, agronomic and managerial factors are of concern to nutritionists in the animal feed industry. Nutritionists usually rely on tabulated feed composition values to formulate least-cost diets, because of the time and expense involved in proximate and amino acid analysis of major feed ingredients (Douglas *et al.*, 1990). Starch granules can differ significantly in size and composition, depending on type and variety of cereals (South *et al.*, 1991).

Rose *et al.* (1993) reported that the chemical composition of a wheat grain varies within cultivars, whereas there is no work that has been done in comparing the chemical composition of hybrid maize and open pollinated maize. The nutritive value of hybrid maize for chicken feeding is well documented, since it is used by commercial feed companies to formulate poultry diets. On the other hand, the nutritive value of open pollinated maize is not known, even though open pollinated maize is still widely used by rural people for poultry feeding. The differences in the chemical composition of these maize varieties may result in birds given diets formulated with these varieties, to perform differently. The differences in composition might be a result of genotype, production location and type and level of fertilizer applied (South *et al.*, 1991). The knowledge of the differences in chemical composition of these maize varieties may help nutritionists in deciding which variety is best for broiler feeding. It is also not known whether different sexes of birds will perform differently to these different maize varieties.

The use of Avizyme 1502 in poultry diets is now commonplace in many countries where the predominant cereal is hybrid maize (Bedford and Morgan, 1996). In most cases Avizyme 1502 has been shown to improve chicken performance and digestibility of nutrients (Bedford, 2000). It is not known whether Avizyme 1502 will show similar results with open pollinated maize or not. The objectives of this study are to: (a) Compare the nutritive value of hybrid maize to that of open pollinated maize for broiler chickens with or without Avizyme 1502. (b) Evaluate the response of the two sexes to the maize types and microbial enzyme supplement.

4.2 Materials and methods

4.2.1 Experimental animals and feeding

Two hundred and forty day-old male and female Ross chicks were reared separately in this study. The chicks were allocated to battery cages in groups of 10 and assigned to one of the dietary treatments. The experimental diets were based on either a local variety of (open pollinated maize), obtained from Kwa-Hlabisa, Northern KwaZulu-Natal province of South Africa, or hybrid maize (Table 4.1). In addition, each of the diets was supplemented with a microbial enzyme, Avizyme 1502 (Finnfeed International, UK) at the rate of either 0 g/kg or 1 g/kg. The experiment was a 2x2x2 factorial design with maize type, sex and enzyme treatment as factors. The treatments were replicated six times. The guaranteed minimum activity of the supplemental enzyme is shown in section 3.2.1. Celite, a source of acid-insoluble ash (Poolbrite S.A. Pty. Ltd), was also included in the diets to enable the assessment of nutrient digestibility. The composition and analysis of the diets is shown in Table 4.2. The diets were fed for 22 days. Food and water were supplied *ad libitum*. The room temperature was managed according to the requirements for brooding chicks between hatch and 21 days.

Table 4.1 *Composition of fresh maize and open pollinated maize (g/kg as fed)*

	Maize type	
Nutrient fraction	**Hybrid maize**	**OP maize**
Dry matter	887.00	899.90
Fat	30.90	40.00
Protein	69.10	82.40
Crude fibre	24.40	19.00
GE MJ/kg	16.40	16.70
AMEn MJ/kg	13.90	14.70
TMEn MJ/kg	14.30	15.10
Calcium	0.06	0.06
Phosphorus	1.70	2.10
Magnesium	0.94	1.01
Threonine	22.4	26.5
Methionine	12.2	18.1
Lysine	21.1	23.3
Phenylalanine	31.2	39.5

[1]OP = Open pollinated

Table 4.2 *Composition (%) and calculated analysis of the diets used in the experiment (as is basis)*

Ingredient	Open pollinated maize diet	Hybrid maize diet
Maize	60.89	60.89
Soyabean isolate	20.00	20.00
Fishmeal 65	6.00	6.00
Sunflower oil	4.27	4.27
L-lysine HCl	0.14	0.14
DL-methionine	0.16	0.16
Vitamin/mineral premix	0.25	0.25
Limestone	1.34	1.34
Monocalcium phosphate	1.20	1.20
Salt	0.26	0.26
Celite	3.00	3.00
Plasterer's sand (filler)	2.50	2.50
Nutrient composition		
Gross energy (MJ/kg)	16.47	16.30
Crude protein	21.09	21.27
Crude fibre	1.93	2.23
Lipids	5.69	6.37
Calcium	1.20	1.15
Phosphorus (digestible)	0.66	0.63
Methionine	0.52	0.46
Lysine	0.32	0.36

In addition, each diet was supplemented with microbial enzyme, Avizyme 1502 at the rate of 0 or 1 g/kg diet.

4.2.2 Assessment of biological response

The chickens were weighed in groups to obtain their body weight at day old and subsequently every 7th day until the end of trial. Food consumption was recorded at the end of each week. The difference between feed supply and feed leftover in troughs was taken as the food intake and the feed intake was corrected for mortalities.

4.2.3 Tissue and digesta collection

Tissue and digesta were collected as described in Chapter 2.

4.2.4 Preparation of tissue, feed and digesta for analysis

Tissue, feed and digesta were prepared as described in Chapter 2.

4.2.5 Determination of nutrients and ileal digestibility of nutrients

Dietary nutrients and ileal digestibility of nutrient were determined as described in Chapter 2.

4.2.6 Determination of tissue protein and the activity of digestive enzymes

Tissue protein and the activities of the digestive enzymes were determined as described in Chapter 2.

4.3 Results

4.3.1 Maize composition

When the two maize varieties were compared, the open pollinated maize used in this study had numerically higher values for fat, protein, energy (GE, AMEn and TMEn), phosphorus, amino acids (threonine, methionine, lysine and phenylalanine) and magnesium than hybrid maize (Table 4.1). Crude fibre content of the open pollinated maize, on the other hand was lower than that of hybrid maize. Calcium was similar in both types of maize.

4.3.2 Biological response

There was no significant effect of all the factors considered and their interactions on feed intake, weight gain and feed conversion efficiency in this experiment (Table 4.3).

Table 4.3 *Feed intake (FI), weight gain, and feed conversion efficiency* (FCE) *of the male (M) and female (F) birds given test diets with(+Aviz) or without(-Aviz) Avizyme 1502*

Maize	FI (g/bird d)	Weight gain (g/bird d)	FCE (g gain/kg FI)
Hybrid	45.85	21.36	466.20
Open pollinated	47.43	22.24	469.90
s.e.d	0.83	0.45	11.77
Sex			
(M)	46.81	21.71	465.91
(F)	46.47	21.89	469.50
s.e.d	0.83	0.45	11.77
Enzyme			
(-) Aviz	46.76	21.74	464.00
(+)Aviz	46.52	21.86	471.42
s.e.d	0.83	0.45	11.77
S.O.V	**P**	**P**	**P**
Maize	0.063	0.056	0.806
Enzyme	0.771	0.787	0.541
Sex	0.690	0.711	0.764

SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significanc

4.3.3 Tissue protein concentration and activities of digestive enzymes

Birds offered diets based on hybrid maize had a significantly ($P<0.05$) higher pancreatic protein content when compared to those given the based on open pollinated maize (Table 4.4). Maize type had no significant effect on the activities of amylase, chymotrypsin and lipase. No interactions between maize, sex, and on the activities of amylase, chymotrypsin and lipase were reported. There was no significant effect of maize, sex and Avizyme supplementation on jejunal protein content nor on the activity of amylase, alkaline phosphatase and sucrase in this experiment when all the factors were taken into consideration (Table 4.5).

Table 4.4 *The effect of maize type and Avizyme 1502 supplementation on the pancreatic tissue protein content and the activities of pancreatic digestive enzymesof male (M) and female (F) birds.*

Maize	Protein[1]	Amylase[2]	Chymotrypsin[3]	Lipase[4]
Hybrid	85.10	2.27	0.61	0.03
Open pollinated	79.00	1.97	0.66	0.03
s.e.d	2.96	0.25	0.09	0.002
Sex				
(M)	82.00	2.27	0.62	0.02
(F)	82.10	1.96	0.66	0.03
s.e.d	2.96	0.25	0.09	0.002
Enzyme				
(-) Aviz	81.50	1.99	0.67	0.03
(+)Aviz	82.60	2.25	0.60	0.02
s.e.d	2.96	0.25	0.09	0.002
S.O.V	**P**	**P**	**P**	**P**
Maize	0.046	0.235	0.652	0.802
Enzyme	0.706	0.286	0.490	0.056
Sex	0.959	0.210	0.679	0.127

[1] = mg/g tissue; [2] = nmole glucose/mg protein/minute; [3] = µmole nitrioaniline/mg µmole nianiline/ mg protein/minute; [4] = lipase unit ;P = Probability of significance
SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502

Table 4.5 *The effect of maize type and Avizyme 1502 supplementation on the jejunum tissue protein and the activity of the jejunal digestive enzymes on male (M) and female (F) birds*

Maize	Protein[5]	Maltase[6]	Sucrase[6]	AP[7]
Hybrid	49.20	2.24	0.03	1.41
Open pollinated	51.00	2.06	0.03	1.23
s.e.d	4.44	0.16	0.002	0.14
Sex				
(M)	50.70	2.04	0.02	1.25
(F)	49.40	2.26	0.03	1.38
s.e.d	4.44	0.16	0.002	0.14
Enzyme				
(-) Aviz	51.60	2.10	0.03	1.34
(+)Aviz	48.60	2.20	0.03	1.29
s.e.d	4.44	0.16	0.002	0.14
S.O.V	**P**	**P**	**P**	**P**
Maize	0.673	0.253	0.793	0.214
Enzyme	0.506	0.519	0.367	0.708
Sex	0.772	0.173	0.111	0.371

[5]=mg/g tissue; [6]= µmole glucose/mg protein/minute; [7]= µmole PNP/mg protein/minute.

AP = Alkaline phosphetase
SOV =Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = Probability of significance.

4.3.4 Ileal digestibility of nutrients

Of all the effects of maize type, sex, avizyme 1502 and their respective interactions on the digestibility of protein, gross energy, phosphorus and calcium only interaction between sex and Avizyme on o digestible GE was significant (P<0.05). With males on Avizyme having a higher GE digestibility than females (Table 4.6). Similar with amino acid digestibility, only the maize x sex x Avizyme interaction had a significant effect (P<0.05).

Table 4.6 *The effect of maize type and Avizyme 1502 supplementation on the ileal digestibility of nutrients in birds (expressed as proportions)on male (M) and female (F) birds*

	Protein				Gross energy				Phosphorus				Calcium			
	OP		Hybrid		OP		Hybrid		OP		Hybrid		OP		Hybrid	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
(-) Aviz.	0.79	0.80	0.79	0.81	0.72	0.74	0.75	0.75	0.37	0.45	0.44	0.44	0.62	0.51	0.62	0.41
(+) Aviz.	0.81	0.79	0.80	0.80	0.76	.72	0.75	0.75	0.46	0.37	0.46	0.44	0.63	0.60	0.62	0.53
s.e.d	0.612				0.022				0.028				0.029			
SOV	**P**				**P**				**P**				**P**			
Maize	0.628				0.062				0.224				0.146			
Sex	0.592				0.781				0.627				0.058			
Avizyme	0.973				0.479				0.750				0.246			
Maize*Sex	0.792				0.561				0.901				0.242			
Maize*Avizyme	0.724				0.812				0.943				0.570			
Sex*Avizyme	0.160				0.045				0.052				0.350			
Ma*Sex*Avizyme	0.371				0.090				0.127				0.328			

OP = Open pollinated maize
SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = plus Avizyme 1502
P = Probability of significance

Table 4.7 *The effect of maize type and Avizyme 1502 supplementation on the ileal digestibility of amino acids (expressed as proportions)on male (M) and female (F) birds*

	Threonine				Methionine				Lysine				Phenylalanine			
	OP		Hybrid		OP		Hybrid		OP		Hybrid		OP		Hybrid	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
(-) Aviz.	0.69	0.72	0.72	0.68	0.92	0.92	0.92	0.92	0.88	0.89	0.89	0.88	0.85	0.85	0.87	0.85
(+) Aviz.	0.73	0.69	0.69	0.72	0.93	0.92	0.92	0.93	0.88	0.88	0.88	0.89	0.86	0.86	0.86	0.87
s.e.d	0.612				0.022				0.028				0.029			
SOV	**P**				**P**				**P**				**P**			
Maize	0.869				0.870				0.556				0.317			
Sex	0.670				0.481				0.939				0.984			
Avizyme	0.565				0.418				0.694				0.509			
Maize*Sex	0.987				0.361				0.626				0.791			
Maize*Avizyme	0.974				0.810				0.933				0.905			
Sex*Avizyme	0.966				0.954				0.656				0.568			
Ma*Sex*Avizyme	0.048				0.248				0.310				0.230			

OP = Open pollinated maize
SOV = Source of variation
(-) Aviz = No Avizyme 1502
(+) Aviz = Plus Avizyme 1502
P = P-Probability of significance
Ma = Maize

4.4 Discussion

4.4.1 Maize composition

The chemical constituents indicate a higher nutritional value for the open pollinated maize compared to hybrid maize used in this study. It is not known why these variations exist, but they may be due to genotype, growing conditions or fertilizers used (South *et al.*, 1991). However, these differences in composition did not translate into any improvement in performance. Further research on the nutritive value of open pollinated maize for broilers is recommended. From the results of this experiment, rural people can be encouraged to use open pollinated maize for their poultry feeding.

4.4.2 Biological response

The present study demonstrated that performance of broilers was not affected by maize type, sex of birds and Avizyme 1502 inclusion in the diet. The observed absence of the effect of Avizyme 1502 on chicken performance is in contrast to reports by Bedford (2000) who reported an increase in chicken performance when the maize-based diet was supplemented with Avizyme 1502. The differences in results may be attributable to maize source and type of processing. The non-significant effect of sex on chicken performance suggests that there are no significant differences in the intestinal macro-environment of the two sexes. Similar observations have been reported by Iji *et al.* (2001c) in research on low-AME wheat fed to male and female broiler chicks. The poor FCE observed in this experiment viz-a-viz commercial expectations (as per Ross Broiler Production Manual-Ross Poultry breeder, 1999) may have been due to the indigestible sand and celite included in the experimental diets.

4.4.3 Ileal digestibility of nutrients

The absence of the significant effect of Avizyme 1502 on ileal digestibility of nutrients was in contrast to our expectations. We had expected the digestibility of these nutrients to be improved by enzyme supplementation, since it breaks down the binding effect of anti-nutritional factors on nutrients, thus making nutrients to be more available for digestion.

4.4.4 Tissue protein content and enzyme activities

The general effects of cereal anti-nutritive factors on intestinal functions were recently reviewed by Iji (1999) He reported that NSP have been known to increase the weight of the gastrointestinal tract tissue weight. In rats this was achieved through an increase in mitotic activity and mucosal growth in the small intestine (Johnson *et al.*, 1981; Pell *et al.*, 1992). The last stage in the digestion of most dietary fractions including carbohydrates and proteins, is affected by membrane-bound enzymes in the small intestine (Kenny and Turner, 1987). Since NSP increase viscosity of digesta, it is most probable that a viscous environment would restrict the access of these enzymes to their dietary substrates, thus constraining digestion. Maize may not have such effects, since it is low in NSP (Choct and Annison, 1990). Avizyme 1502 may not have any effects on endogenous enzymes but appears to work independently. In the present study it had no effects on animal productivity or digestive functions.

4.5 Conclusions

Chemical constituents demonstrate that open pollinated maize has higher nutrient value than hybrid maize but this did not improve performance. Since these maize varieties were from different sources, the variations might be as a result of genotype, growing condition or fertilizer used (South *et al.*, 1991). Avizyme 1502 and sex of birds did not have a significant effect on any of the variables measured in this experiment. Microbial enzymes are less effective on diets with low levels of anti-nutritive factors. Avizyme 1502 appeared to have no effect on endogenous enzymes, but appears to work independently. Further research on the nutritive value of open pollinated maize for broilers is recommended. From the results of this experiment, small-scale farmers can be encouraged to use open pollinated maize for poultry feeding.

CHAPTER 5

GENERAL DISCUSSION

5.1 Effects of different factors on different variables measured

(a) Effect of tannins on productivity

Tannins had a negative effect on the biological performance of broilers. The digestibility of nutrients was also negatively affected by tannin inclusion in the diet. Since tannins are anti-nutritive factors, and anti-nutritive factors are well known for the increasing viscosity of digesta (Fengler *et al.*, 1988; Choct and Annison, 1992), it is most probable that a viscous environment would restrict the access of membrane-bound enzymes to their dietary substrates and as such constrain digestion. It is therefore advisable for poultry producers not to use ingredients high in tannin content for broiler feeding. Supplementation with dietary enzymes (Avizyme 1502) can overcome the anti-nutritional effect of tannins to a limited extent.

(b) Effect of maize drying temperature on productivity

Drying maize had a positive effect on the biological performance of broilers. Drying of maize also increased the ileal digestibility of some nutrients. Drying temperatures above 95°C had a negative effect on maize quality for broilers, possibly because of denaturation of nutrients at high temperatures. Poultry farmers should continue drying their maize to improve the nutritive value for broilers, but care should be taken not to dry it at very high temperatures (in excess of 95°C).

(c) Comparisons between hybrid maize and open pollinated maize

The chemical constituent indicators point towards a high nutritional value for open pollinated maize compared to hybrid maize in this study. Whilst the performance traits measured (feed intake, weight gain and feed conversion efficiency) generally reflected the apparent high nutritive value of the open pollinated maize, the effect was not statistically significant. However, the findings indicate the nutritional potential of a maize genotype that has not been subjected to much scrutiny by animal nutritionists. Further research on the nutritive value of open pollinated maize genotypes grown by small-scale farmers is recommended. Furthermore, small-scale farmers can be encouraged to continue feeding open pollinated maize to their chickens, and thus to continue conserving this plant genetic resource.

(d) Effect of microbial enzyme (Avizyme 1502)

Avizyme 1502 had the most positive effect on the birds that were given diets containing tannins. As an anti-nutritive factor, tannins are known to bind nutrients. The non-significant effect of Avizyme 1502 on diets containing maize can be attributed to the low level of NSP in maize. It can thus be concluded that microbial enzymes are more effective when added to diets with high levels of anti-nutritive factors. It will be pointless for feed companies to add dietary enzymes to broiler diets based on high quality ingredients.

5.2 Direction for future research

Future research could focus on:

- Processing methods, other than microbial enzyme supplementation, to improve the biological value of high tannin diets, such as sorghum-based diets (e.g. different levels of heat treatment, steam, direct heating or fermentation).
- Comparing the effect of Avizyme 1502 supplementation on broiler performance during the starter phase and finisher phase of growth.
- Determining what effect heat (different drying temperatures) can have on the nutritive value of open pollinated maize for broiler feeding.

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