UNIVERSITY OF NATAL

INFLUENCE OF COMPOSITION AND STORAGE CONDITIONS ON DIET QUALITY AND PRODUCTIVITY OF BROILER CHICKENS

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By

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Summary

Two experiments were conducted to assess the effects of varying storage temperature and relative humidity (RH) on feed quality and nutritive value of such diets when fed to broiler chickens. The diets used in experiment two were higher in lipids than those used in experiment one. All other features of the diets tested in the two experiments were identical. Diets were also supplemented with or without inhibitor during storage. Prior to feeding, the diets were also supplemented with or without a fungal detoxifier.

Laboratory analysis showed that the diets contained *Aspergillus spp, Penicillium spp* and *Fusarium spp* with no toxins identified. Storage at high temperature (30°C) over one month increased dry matter (DM) content by about 1%, which was accompanied, by an increase of between 3.5-5% in crude protein (CP) as well as 3-4% fat (Experiments One and Two). After two months of storage, high storage temperature decreased DM content by about 1.6%, which resulted in a decrease in the levels of CP and fat in most of the diets (Exp. One). In experiment two, an increase of about 0.5% in the DM content was observed in diets stored at low temperature (15°C) followed by an increase of about 3% in fat content. Variable changes were observed in the micro-mineral contents. In experiment one, there was an increase in Fe and Zn content after one month, followed by variable decreases in the second month, while in experiment two, there was a decline in the concentrations of Fe, Zn and Cu by about 21, 16 and 26%, respectively throughout the storage period.

Rancidity developed with time in all the diets but there was a tremendous reduction ($p<0.001$) in the rate of oxidation by 15% and 20% with supplemental inhibitor (antioxidant) in experiment one and two, respectively but no reduction in free fatty

acid (FFA) content. High storage temperature (30°C) and RH (80%) increased FFA content from 3.5 to about 15% in experiment one ($p<0.001$, $R^2 = 0.73$) and to about 17% in experiment two ($p<0.001$, $R^2 = 0.84$) during the storage period of 60 days. Storage at low temperature (15°C) and RH (50%) similarly decreased peroxide value (Pv) by about 16% in experiment one ($p<0.001$, $R^2 = 0.84$) and in experiment two by 25% ($p<0.001$, $R^2 = 0.89$) over time. Inclusion of the antioxidant decreased the concentration of Pv by about 10% in experiment one ($p<0.001$) and by 20% in experiment two ($p<0.001$).

Feed intake of birds was unaffected. Storage temperature did not influence body weight (Experiment one) but in experiment two, high feed storage temperature decreased ($p<0.001$) body weight by about 3%. Supplementation of diets with the inhibitor improved ($p<0.001$) body weight only in experiment two. Further supplementation with the detoxifier markedly improved ($p<0.01$) body weight in both experiments. Low RH increased feed conversion efficiency (FCE) by 6% in experiment one ($p<0.001$) and in experiment two ($p<0.01$). Improvements ($p<0.05$) in FCE were observed on diets stored at low temperature in experiment one and with further supplemental detoxifier, ($p<0.01$). No significant differences were noticeable in body weight or in FCE in experiment two. Mortality was unaffected by treatments.

The relative liver weight of birds was markedly reduced as a result of low storage temperature and RH. Dietary supplemental inhibitor or detoxifier reduced ($p<0.001$) liver weight between 8 and 10% in both experiments. On the other hand, heart weight was unaffected by storage conditions. However, in experiments one and two, the detoxifier caused a reduction ($p<0.001$) of 11% in heart weight unlike the inhibitor.

Proventriculus weight was reduced ($p<0.05$) by feeding diets stored at low RH in experiment one by 4%, while no effects were observed in experiment two. Both studies showed no changes in the proventriculus weight when the inhibitor and detoxifier were added. Storage at low temperature and RH in experiment one caused a significant decrease ($p<0.01$) of 4% gizzard weight unlike in experiment two. The inhibitor reduced gizzard weight by about 7% in experiment one ($p<0.001$) and experiment two ($p<0.05$). Further supplemental detoxifier decreased gizzard weight in experiment one ($p<0.01$) and two by 6% ($p<0.001$). Spleen weight was not affected by any of the treatments except in experiment one, where the weight of the spleen was reduced ($p<0.05$) by detoxifier from 0.13- 0.12g/100g body weight (7.7%).

Storage conditions did not affect the biochemistry of serum obtained from the chicks in experiment one, while in experiment two, high temperature decreased ($p<0.05$) total phosphorus from 3.4-2.9 mmol/l and high RH decreased ($p<0.05$) serum protein (3.3-3.1g/100ml) as well as globulin (1.74-1.58g/100ml). Supplemental inhibitor increased serum protein ($p<0.05$) by 6% and globulin ($p<0.01$) by about 12% (experiment one) and albumin ($p<0.05$) by 7% (experiment two). Further supplementation with the detoxifier increased ($p<0.001$) phosphorus by about 3.5% (experiment one), while in the second experiment, serum phosphorus and albumin were increased ($p<0.05$). No signs of 'rubbery' bones were observed in birds in these studies. Furthermore, there were no observable changes as a result of storage conditions or addition of inhibitor in diets. However, it was observed that supplemental detoxifier increased bone ash from an average of 51.2 to 51.8% in experiment one and in experiment two, 50.6-52.5%.

The results of the studies indicated a development of feed contamination (quality loss) over time with tremendous rate of increase observed in diets devoid of the preservatives (antioxidant and mold inhibitor). Further supplementation of the detoxifier markedly improved productivity as well as some of the serum biochemical parameters measured.

Declaration

I Njobeh Patrick Berka hereby declare that the research reported in this thesis is the result of my own investigations, except where acknowledged, and has not, in its entirety been previously submitted to any University or Institution for publication or degree purpose.

Sign .....

I Dr Iji, P. A., Chairman of the Supervisory Committee, approve release of this thesis for examination.

Sign .....

Dedication

This thesis is dedicated to my parents

Mr Berka Soh Sylvester

And

Mrs Berka Felicitas nee Kongran

Acknowledgement

I am greatly indebted to my supervisor Dr Iji, P. A. for his timely acceptance as my supervisor, providing professional guidance, constructive criticisms and immediate responses to my write-up and above all, the sacrifice he made in having sleepless nights to make sure I graduate with my peers. To him, I will say, GOD BLESS YOU!"

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CHAPTER ONE

1.0 General Introduction

For livestock feeds to be constantly available in a farming enterprise, it is required that large amounts of feeds are produced and stored for a considerable period of time. Feed millers also buy ingredients during the season of production when prices are low, and such ingredients could be stored for a considerable length of time before they are used. Storage of feeds is generally associated with spoilage or loss in quality. The economic implication resulting from loss in feed quality cannot be overemphasised. Loss in feed quality depends on the nutrient composition as well as storage environmental conditions. A large amount of work has been reported in this area (Coker, 1979; Virginia, 1989; Blaha *et al.*, 1990; Valarezo *et al.*, 1997).

Another area in which research has been undertaken is in the use of preservatives in preventing spoilage and sorbent materials as toxin-binders (Bailey *et al.*, 1998). In most of these studies, laboratory-produced toxins have been used. Studies in which the natural fungal growth pattern is monitored in diets during storage are limited. Furthermore, a search of information on related literature failed to detect any investigation to simultaneously assess the combined effects of factors involved in spoilage and preservation of feeds during storage as well as determine the extent to which such diets affect animal performance in the African continent.

1.1 Objectives of the study

1) Determine feed quality under various storage conditions over time;
2) Determine the efficacies of a mold inhibitor and an antioxidant in preventing feed spoilage;

3) Evaluate the efficacy of toxin-binder in preventing mycotoxicosis, and

4) Determine the influence of stored feeds on the performance of broilers.

CHAPTER TWO

2.0 Literature Review

2.1 Introduction

It is desirable that the review on the present topic be treated in two sections. Firstly, it is imperative that a clear understanding of the concepts of livestock feed and its components are identified and discussed. This part of the review also focuses on feed contamination, specifically stating the various types, causes and consequences of contamination, with particular reference to fungi.

The last part of the review critically examines the measures that could be adopted to redress spoilage. The role played by hazard analysis critical control point (HACCP) in the feed industry will be clearly stated.

2.2 Animal feeds

2.2.1 Definition and concept

Any material eaten by an animal as part of its daily ration is feed (Arthur, 1979). McDonald *et al.* (1995) defined feed as any substance or material, which after ingestion by animals is capable of being digested, absorbed and utilised by the body of the animal. Feed must be wholesome, acceptable and palatable. Josephine (1996) also defined feed as any substance that is capable of nourishing the body by supplying any or all of the following nutrients: protein, fat, minerals, vitamins and water for growth, tissue repair, maintenance and reproduction.

2.2.2 Components of animal feeds

Feed is generally made up of those components (nutrients) that are capable of being utilised by the body. Arthur (1979) defined nutrient as any chemical element or compound having specific functions in the nutritive support of animal life. Livestock nutrition is a matter of providing animals with the correct amounts of these chemical substances essential for the various body activities. The amount of nutrients required varies from species to species, age of the animal, physiological status and the purpose for which the animal is kept. Feeds are classified into:

2.2.2.1 Carbohydrates

Carbohydrates contain carbon, hydrogen and oxygen with H_2 and O_2 in the same proportion as water. Carbohydrates are broadly divided into monosaccharides (e.g. glucose and fructose), disaccharides (e.g. sucrose and maltose) and polysaccharides (e.g. starch and lignin). Monosaccharides are formed from plants by photosynthesis in which carbon dioxide reacts with water in the presence of sunlight absorbed by chlorophyll (Cheeke, 1999).

$$6\ CO_2 + 6\ H_2O + ADP \longrightarrow C_6H_{12}O_6 + 6\ O_2$$

Dissacharides are produced by polymerisation of two monosaccharide molecules while polysaccharides are formed by a combination of three or more molecules of monosaccharides (Kellems and Church, 1998b). Most cereal and legume plants store energy in their seeds in the form of starch, which is the major component of most monogastric rations.

Carbohydrates supply energy, required in a living cell to build muscle tissues, for muscle contraction as well as transport of other nutrients. Carbohydrates are the most

important components that make up the bulk of livestock feed (Ckeeke, 1999). Sources of carbohydrates include cereal grains, roots, tubers, sugar plants, molasses, plant leaves, etc. Maize is the most widely used of all energy sources in monogastric feeding in the tropics and southern Africa, which makes up about 50% of the total feed composition. Energy sources are usually very limited in amino acids, particularly in the essential amino acids such as lysine, methionine and tryptophan (McDonald *et al.*, 1995; Cheeke, 1999) and so must be supplemented with protein for most classes of livestock.

2.2.2.2 Fats/oil

These are substances of plant and animal origin, insoluble in water, but soluble in organic solvents and contain carbon, hydrogen and oxygen. Dietary fats are the most concentrated source of energy (Josephine, 1979; Michael, 1987) providing about 2.25 times as much energy per kg as carbohydrates (Arthur, 1979; Kellems and Church, 1998b). Fats provide the essential fatty acids such as include oleic, linoleic and linolenic acids (Michael, 1987), which are the major components of the cell membrane and also play a significant role in kidney function and reproduction. Fats also help in the transport of the fat-soluble vitamins A, D, E and K. Gurr (1984) indicated that plant lipids are also good sources of vitamins A and E. Other functions of fats are to improve palatability, reduce dustiness, as binders and lubricants in the feed industry (Kellems and Church, 1998b). Sources of fats/oil include legume seeds, vegetable seeds, leaves and animal by-products such as eggs, meat and milk. Fat inclusion in diets is less than 10% due to its instability when subjected to heat.

2.2.2.3 Proteins

With the exception of water, protein is found in the highest concentration of any nutrient in all living organisms including animals (Kellems and Church, 1998a). They are complex organic compounds found in all living cells with high relative molecular weights. Proteins are broken down with the aid of enzymes as catalysts to amino acids. Amino acids are organic acids containing one or more amino groups (NH_2), which are classified into essential (indispensable) and non-essential (dispensable) amino acids. Essential amino acids are required in the diets because they cannot be synthesized by the body while non-essential amino acids are also important but can be synthesized in adequate amounts from other amino acids and suitable carbon compounds.

Protein plays a very essential role in that it is the structural component of the cell, regulates body metabolism, as enzymes and some hormones (reproductive), and is required for growth and replacement of worn-out tissues. Excess protein in the body may be converted to fats and stored in the adipose tissues (Cheeke, 1999). Protein can be categorised into true protein and the non-protein nitrogen (NPN). The non-protein nitrogen includes the compounds that are not classified as true protein but are metabolised to amino acids (Kellems and Church, 1998c).

Examples of NPN include urea, amides, ammonia, amines, amino acids and biuret. Sources of protein include by-products of oilseeds, meat and bonemeal, milk, blood meal, fish meal, feather meal, poultry litter, etc. The most widely used protein source in the world is soybean meal (Arthur, 1979; Geoffrey, 2001).

2.2.2.4 Vitamins

According to McDonald *et al.* (1995), vitamin was derived from "*vital amines*" which describes these accessory food factors to contain amino-nitrogen. Arthur (1979), McDonald *et al.* (1995) and Kellems and Church (1998a) defined vitamins as organic compounds required by animals in small amounts for normal growth and maintenance of animal life. They contain carbon, hydrogen and oxygen and in addition, several contain nitrogen.

They are classified as fat-soluble (A, D, E and K) or water-soluble (B-complex and C) according to their solubility in fats and water, respectively. Vitamins have diverse functions depending on the type. They mainly regulate body functions. Sources of vitamins include liver, kidney, yellow corn, milk, vegetables, fruits, premix, etc. Vitamins are micronutrients, required in relatively small amounts but deficiency in one or more of them can result in poor performance of the animal. Arthur (1979) and McDonald *et al.* (1995) indicated that higher levels of vitamins in the body are toxic leading to hypervitaminosis (a disease condition in animals as a result high concentration of vitamins, depending on the type of vitamin). Effects of both low and high levels of the individual vitamins on animals are discussed at length by Church and Kellems (1998b).

2.2.2.5 Minerals

These are inorganic components of the diet required in small amounts like the vitamins that provide an essential function in the animal's metabolism. Requirements for minerals vary from species to species, age of the animal and purpose for which they are kept. For example, laying chickens require about 3-3.5% calcium in their diets, while

broiler birds require about 0.5% (NRC, 1994). They may be broadly divided into micro and macro elements based on the concentrations found in an animal's body (Kellems and Church, 1998a; Church and Kellems, 1998b). Micro-minerals are required in relatively small amounts (below 100ppm body concentration) and include iron, copper, zinc, iodine, selenium, manganese, fluorine, cobald and molybdenium. On the other hand, calcium, phosphorus, potassium, sodium, sulphur, chlorine and magnesium are macro elements required in amounts exceeding 100ppm body concentration.

Some minerals occur as structural components, forming the skeletal part of the body, regulate body processes, for milk production, while others act as enzyme activators by binding at site that of the enzyme that is not active thereby stimulating it to catalyse any substrate present. Others play a fundamental role in cell metabolism. Church and Kellems (1998c) have detailed information regarding mineral supplementation in diets. Sources of minerals are bone meal, fish meal, iodised salt, eggshell, vegetables, seeds, milk and mineral premix.

2.2.2.6 Water

It is the simplest but most important of all substances in feeds, containing hydrogen and oxygen. Its concentration ranges from about 10% in air-dry feeds to over 80% in fresh green forage (Arthur, 1979). Water is vital to the life of the animal occupying about 71 - 73% body weight (Kellems and Church, 1998a) and performs a variety of functions ranging from maintenance of body temperature to transport of nutrients and excretory materials. While experts on livestock production are aware of this fact, feed producers are much more worried about feed storage because, it encourages feed spoilage. Sources of water include drinking water, metabolic water and water from feed. The role of water

in nutrient absorption, metabolism and deficiency syndromes is fully elaborated by Arthur (1979), Cheeke (1999) and Kellems and Church (1998a).

2.2.3 Importance of animal feeds

Animal feeds are formulated to meet the nutritional requirements of farm animals. The domestication of animals is for various purposes. While some are kept for egg or meat production, others are raised for milk production. The nutritional quality of feeds offered to animals will determine to a great extent their productive performance. Nutritional research has played a prominent role in animal production where information is provided on the planning and analyses of diets. Feed science provides information on the link between nutrition and health whereby certain nutrients are used in corrective and preventive medicine.

The purpose of a farm animal is to convert feed into some form of animal product (human food). The efficiency of conversion will vary as a function of species and age of the animal on the one hand, and on the other, as a function of the nutrient composition of the feed. Feed is a structural material for building and maintaining the body structure, source of energy and for regulating body processes such as milk production (Arthur, 1979 and Robert, 1979).

2.2.4 Feed processing

Broiler feeds in one way or the other, are subjected to some form of treatment before they are ready for consumption. Method of processing is determined by the type of animal destined to consume the feed, type of feed, cultural background, equipment, raw materials available and the level of livestock production. Some feeds are processed to

change their form, improve palatability, improve the nutritional value, for uniformity and also to control the microbial load or remove poisonous chemicals for proper preservation (Crampton and Harris, 1969; Stephenson, 1972). During feed processing, certain nutrients such as vitamins and amino acids are destroyed or may be contaminated by microbes. Processing is actually one or a combination of heating, irradiation, microwaving, grinding or chopping into smaller particles, cooking, pelleting, fermenting, treating with urea or alkali, mixing etc.

Feed producers are faced with many challenges where there is a shift in focus from feed production to management of food safety. This shift dates back to 1969 when the Swan Report suggested that the use of antibiotic in animal feeds can induce bacterial resistance (Lyons, 1998). Feed industry has suffered considerable loses caused by food safety problems (Lyons, 2000) due to spoilage of raw materials (feed ingredients) as well as by-products (feeds) caused by microbial contamination (Paster *et al.*, 1985; D'Mello *et al.*, 1999). The feed producers are increasingly aware that they target animals by providing feeds of high nutritional quality for optimal performance as well as to ascertain product quality and safety for human consumption.

2.2.5 Feed storage

Having feed available for livestock throughout the year requires that large amounts of feeds are produced and stored for a considerable period of time. This is due to the fact that most crops used as feed ingredients are seasonal in nature and must therefore be subjected to storage from harvest to feeding (Arthur, 1979). Production of mixed feeds inevitably entails storage of both the raw materials and finished products. McEllhiney

(1985) stated that storage of feeds is generally associated with spoilage, especially if the material is rich in fats and moisture.

The greatest bulk of feed spoilage occurs during storage influenced by the nutrient composition of the diet. An excess amount of moisture will encourage mold growth and subsequent production of toxins. High fat diets are subject to higher levels of loss due to rancidity. The method designed for storage of animal feeds will be determined government policies, chemical composition of the diet, type of feed, equipment available, availability of preservatives and the environmental conditions.

2.3 Feed contamination and spoilage

2.3.1 Definition and concepts

The presence or introduction of any material or substance of nutritional importance into the feed, which will effect spoilage or be harmful to the animal after ingestion, is referred to as contamination. Microbes, toxins and inedible materials, such as metals, fall within this category. Lowe and Kershaw (1995) indicated that feedstuffs and feeds should remain stable and safe for consumption over a specified shelf-life. A report on AGRI-FACTS (2003) indicated that forage and cereal contamination takes place in the field (plant-pathogenic), during processing and storage of harvested products and feeds (spoilage). Contamination of feeds results in spoilage, which may take many forms such as mold growth and development, production of mycotoxins, development of off-flavour and unpleasant odour (rancidity) and in addition, loss in nutrients.

Insects, nematodes and rodent infestation also contaminate feeds as they feed and defecate on these materials thus exposing them to spoilage. Grains infested with mold

may contain toxic fungal metabolites called mycotoxins (Herrman, 2002), which could contaminate a whole batch of feed during processing in the feedmill. If the batch feed has a toxic level beyond the critical control point (LD_{50}), the whole lot is rendered unfit for consumption resulting in severe losses to the industry. Maximum levels of mycotoxins in diets vary from species to species, age and also the type of toxin. In growing broilers, aflatoxin level of 20ppm (Meronock and Concibido, 1996), while Leeson *et al.* (1995) cited by Placinta *et al.* (1999) indicated 140mg/kg deoxinivalenol (DON) and 3.6-5.25mg/kg body weight of T-2 toxin. Hinton (1993) concluded that fungi are the principal cause of spoilage of animal feeds, particularly legumes and cereals.

2.3.2 Aspects of feed contamination and spoilage

There is considerable evidence that livestock feeds are frequently subjected to various forms of contamination and spoilage. Each aspect of contamination will be discussed in detail.

2.3.2.1 Insect and rodent infestation

These are the most important causal agents in the loss and damage of stored feed concentrates (Francis and Wood, 1980). During storage, insect pests and rodents attack these products with equal persistence and feed on them with a resultant loss in nutrient quality of the feed. In addition, rodents may defecate or urinate on feeds thus increasing the moisture content, thereby encouraging mold growth. The products of excretion may even contain toxic substances that are poisonous to the animals.

Insects and rodents in most cases, act as agents of feed contamination by transferring fungi spores from contaminated feeds to recontaminate others. They may act as promoter as promoters of spoilage by either increasing the moisture content of the temperature thus encouraging spoilage. At high temperatures, they can multiply and reach an alarming proportion (Michael, 1987) because of the high survival rate of their offsprings. Thus, they burrow into the feed in search of food and shelter thereby increasing the temperature within it (Francis and Wood, 1980). This rise in temperature favours mold growth and the production of mycotoxins (Blaha *et al.,* 1990; Njobeh, 1997; Valarezo *et al.*, 1997 and Virginia, 1989). In addition, lipids in such feeds may undergo hydrolysis and oxidation leading to spoilage which invariably reduces quality.

2.3.2.2 Rancidity

Fats and oil of plant and animal origin are concentrated sources of energy, which are widely used in feed production. However, they are very unstable and subject to rancidity. The word rancidity is derived from a Latin word "*rancidus*" meaning *stinking* (Sanders, 1989), the taste or smell of rank stale fat. Rancidity is one of the major problems associated with the use of fats in animal feeds. It results in the development of off-flavour during storage.

There are two types of rancidity of economic importance depending on the type of chemical reaction. Oxidative rancidity according to McDonald *et al.* (1995), Francis and Wood (1980), Enser (1984), Hamilton (1989) and Cheeke (1999) is the process by which polyunsaturated fatty acids are oxidized to produce various peroxides. Sanders (1989) indicated that this reaction is accelerated by heat, light, water and certain metals (iron and copper). Oxidation that occurs in feeds can be equated to that which occurs in

metals causing rust and deterioration (Rumsey, 1978). Hydrolytic rancidity is one in which fats are hydrolysed in the presence of an enzyme, lipase to produce free fatty acids and glycerol giving a soapy off-flavour (Francis and Wood, 1980). The chemistry of fat decomposition is fully described by Hamilton (1989).

The rate of fat deterioration in feeds will largely depend on the concentration of polyunsaturated fatty acids (degree of unsaturation) (Sanders, 1989; Berger, 1989). Generally, unsaturated oils are much more susceptible to peroxidation than the saturated oils (Berger, 1989). The higher the degree of unsaturation, the more likely the oils become unstable (Coppen, 1989). Biological membranes with relatively high concentrations of polyunsaturated fatty acids (PUFA) are much more susceptible to lipid peroxidation (Ramezanzadeh *et al.,* 1999). This is because fatty acids are attacked by O_2 at the C-C double bonds of unsaturated fatty acids with the initial formation of peroxides (Coppen, 1989).

Ruiz *et al.* (2000) noted that the rate of peroxide production in meat will depend on the fat content, presence of pro-oxidants (haem or non-haem iron), storage conditions (Berger, 1989) and presence of antioxidants. High temperature and relative humidity conditions of storage will increase the rate of lipid oxidation. In addition, it is obvious that peroxidation cannot take place in the absence of oxygen. Further details on rancidity as an aspect of spoilage are provided in the proceeding sections (Section 2.3.3).

2.3.2.3 Microbial attack and production of mycotoxins

Fungi are well adapted for growth and development over surfaces and through solid materials. The enzymes they secrete break down the available nutrients, which are then transported to the actively growing hyphae (Smith and Moss, 1985). Forage and cereals are materials often attacked by microbes during favourable conditions (temperature and humidity), within which their spores grow and multiply. Blaha *et al.* (1990) stated that an air temperature of about 30°C or above and a relative humidity of 80 – 100% are ideal conditions for mold growth. Fungi in agricultural commodities can be identified in a culture medium such as Ohio Agricultural Experimental Station Agar (OAESA) or Potato dextrose agar (PDA).

Marais (2000) reported an estimate of 70,000 species of fungi known to exist. However, fungi of economic importance recognised as plant pathogens (field fungi) or saprophytes (storage fungi) are the Fusarium, Aspergillus, Penicillium (Blaha *et al.*, 1990; Lin and Chen, 1995; Piva and Fabio, 1999; Jelinek *et al.*, 1989, cited by Raju and Devegowda, 2000) and Alternaria (Dutton and Kinsey, 1996; Placinta *et al.*, 1999), which produce toxic secondary metabolites called mycotoxins.

The word mycotoxin comes from the Greek word "*mykes*" meaning fungus and "*toxicum*" meaning *toxin* or *poison* (Hundley, 2001). Mycotoxins occur worldwide in a variety of feedstuffs. About 300 – 400 mycotoxins are known (Betina, 1989; Huff, 1992; Piva and Fabio, 1999; Herrman, 2002) but of most concern based on their occurrence and toxicity are aflatoxins, deoxynivalenol (DON), ochratoxin A, fumonisins, zearalenone (D'Mello and MacDonald, 1997; D'Mello *et al.*, 1999; Piva and Fabio, 1999; Devegowda and Raju, 2000; Marrais, 2000; Newman, 2000; Herrman, 2002) and claviceps (Diekman and Long, 1988; Ledoux and Rottinghaus, 1999).

Occurrence (Devegowda *et al.*, 1998) and incidence of mycotoxins vary from one region to the other. These will depend on the climatic conditions of the area that are considered favourable for growth of fungi. The occurrence of mycotoxins in various commodities throughout the world is presented in Figure 2.1.

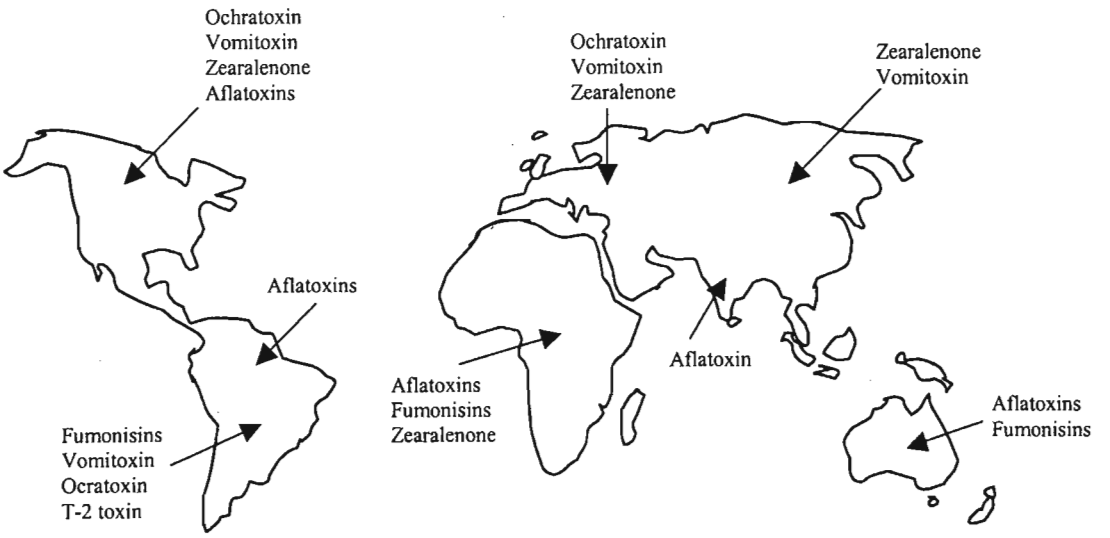

Figure 2.1 Geographic occurrence of mycotoxins (*Source: Devegowda et al., 1998*)

An exhaustive investigation on the occurrence of fungi and mycotoxins in animal related feedstuffs between 1984 and 1993 in South Africa was conducted by Dutton and Kinsey (1996). Data obtained confirmed that aflatoxin and trichothecens were the most predominant mycotoxins with an incidence of over 14 and 10%, respectively. In this survey, maize, the most widely used energy source in the South African feed industry and maize-containing feeds had Fusarium incidence of 32%. The distribution of incidence of mycotoxins in commodities in this survey is presented in Table 2.1.

Table 2.1 **Incidence of mycotoxins in South African Agricultural commodities from 1984 to 1993 (*Source: Dutton and Kinsey, 1996*)**

	Mycotoxin (No. Samples positive)[a]						
Commodity	**AF[d]**	**TH[d]**	**KA[d]**	**FB_1[d]**	**ZN[d]**	**PA[d]**	**other[e]**
Maize	35	56	17	10	21	3	6
Other cereal	7	4	0	0	2	1	0
Oil seed	37	2	0	0	3	1	0
Animal feed	57	69	9	14	31	2	7
Poultry feed	46	26	7	20	7	4	7
Fish meal	4	0	0	0	0	0	0
Animal tissue	0	0	0	0	0	0	
Forage[b]	6	1	0	0	0	0	2
Soyabean	9	0	0	0	0	1	0
Miscellaneous[c]	18	11	1	0	3	21	2
Total	229	169	34	44	67	33	20

[a] Total number of samples assayed = 1602. [b] includes: hay, Lucerne, and poultry litter. [c] includes: bagasse, beer, brewer's grain, dry beans and food. [d] includes: aflatoxin (AF), trichothecene (TH), kojic acid (KA), fumonisin B_1 (FB_1), zearalenone (ZN), patulin (PA). [e] includes: gliotoxin (1), cyclopiazonic acid (1), penicillic acid (50), oosporein (6), tenuazonic acid (2), stergmatocystin (4).

Bracket (1992) indicated that quality and safety of fresh produce depend on their microbiological flora and every step from production to consumption will influence its microbiology. Feed microbial population will increase with improper handling and poor storage. Microbes require the presence of available water for growth and metabolism (Conchello, 2002). Clark (2003) concluded that the incidence of mycotoxin contamination varies between feedstuffs and from region to region. Fumonisins are common in maize, aflatoxins in peanuts, ochratoxin A is found to be problematic in Scandinavian countries while DON are found in wheats in the United States of America, Canada and Europe (CAST, 2003).

Hamilton (1985), cited by Lin and Chen (1995) indicated that the frequency of aflatoxin contamination was moderate at ingredients receiving point, increased to 52% at manufacture, and finally to 91% at feeding. This shows that mycotoxin contamination takes place at each stage of feedstuffs and feed handling (from production to feeding).

2.3.2.4 Presence of inedible substances

It is not uncommon to find foreign materials in feedstuffs. Such materials may play a role in influencing feed quality or may be harmful to animals. Cereal grains in the field could be harvested along with plants such as lupins, Senecio species, leafy spurge and larkspur which contain some toxic substances other than mycotoxins, e.g. alkaloids, tannins, cyanides and perloline (Kellems and Church, 1998b). These plants are poisonous and carcinogenic by nature and so their presence could render feeds unsuitable for consumption.

Some particles of metals, stones or polythene bags incorporated without knowledge into the feed during harvesting or processing are liable to reduce feed quality or cause severe damage to the animals if consumed. In addition, remains of dead animals in feeds may be a predisposing factor to disease.

2.3.3 Factors that influence feed deterioration

There are quite a number of factors responsible for the undesirable changes in feeds. These factors include climate, chemical composition of the diet, improper feed handling and length of storage. Each of the factors stated above will be critically examined.

2.3.3.1 Temperature and relative humidity

It is important to note that there is an inverse relationship between feed stability and environmental temperature and humidity. Shelf-life of feed is shortened by high temperature and relative humidity. Ambient conditions (temperature and humidity) do not only influence the rate at which chemical changes may take place, but also the growth of insect pests and fungi (Francis and Wood, 1980, Farrar and Davis, 1991, cited by D'Mello and Macdonald, 1997). This is because high temperatures and relative humidity provide ideal conditions for growth and development of molds (Virginia, 1989; Blaha *et al.*, 1990; Valarezo *et al.*, 1997) with possible production of mycotoxins.

Apart from the rapid proliferation of molds in feeds at adverse environmental conditions, fats are also known to be unstable, undergoing one or both of oxidative and hydrolytic rancidity. This proceeds to much higher levels when temperature exceeds 25^0C. Van den Berghe *et al.* (1990) reported that in the tropical regions where conditions are always favourable for feed spoilage, animal feed storage may cause problems related to oxidation and mold development. Coker (1979), Meronuck and Concibido (1996) and Valarezo *et al.* (1997) identified optimal temperature range for aflatoxin production to be 25 – 30°C. Aflatoxin production in feeds occurrs at 10 – 13% moisture and 70% relative humidity and these conditions are often regarded as threshold safety level for mold growth (Jones *et al.*, 1982; Beuchat, 1991; Smith *et al.*, 2000)

2.3.3.2 Chemical composition of the diet

According to Njobeh (1997), the shelf-life specified for animal feeds is variable, depending on the chemical composition of the diet. From a study conducted on factors that affect spoilage of animal feeds, Njobeh (1997) reported that fat, moisture and to a

lesser extent, protein content would determine the shelf-life of livestock feeds. This report is in agreement with Paster *et al.* (1985) who stated that a diet of higher nutritional value would be, under certain climatic conditions, more sensitive to fungal spoilage and may also affect the efficiency of mold inhibitors. High protein may not significantly influence rancidity, but may encourage microbial growth due to its nitrogen supply. In addition, diets containing high concentration of PUFA are known to have a shorter shelf-life.

It is necessary to mention that moisture content *per se* is not the only factor determining feed spoilage, but the water activity ($_a$w) as well (Van Schothorst and Brooymans, 1982; Smith and Moss, 1985; Lowe and Kershaw, 1995; Conchello, 2002). Water activity as defined by Lowe and Kershaw (1995) is a measure of partial pressure of water vapour in equilibrium with the substrate relative to that of pure water at the same temperature. Water movement within the tissues encourage growth of microbes.

Adams (1985) concluded that developing molds use nutrients to satisfy their own metabolism and mycotoxin production may occur. Betina (1989) reported that ochratoxin A produced by *Aspergillus ochraceus* and *Penicillium viridicatum* increased with increase in protein level. It has been observed that certain fat-soluble vitamins in feeds in addition to fats themselves are subject to oxidation (Waldroup *et al.*, 1981).

2.3.3.3 Length of storage period

Length of storage period is another important factor that influences feed deterioration. Once a feed is processed, it is assumed that it can be stored at least for a short time without detriment, it is therefore required that storage microbes need to be kept in mind

as they will determine both the types and final population of microbes present (Brackett, 1992).

Considering the fact that fat-soluble vitamins, fats and moisture influence feed quality, it must therefore be borne in mind that diets that are high in fat and moisture are intrinsically more unstable and their instability increases with age (Paster *et al.*, 1985; Lin *et al.*, 1989; Njobeh, 1997; Khalil and Mansour, 1998;), with or without preservatives. Sanders (1985) confirmed that lipid peroxide concentrations increase with length of storage period with or without antioxidant. It must therefore not be assumed that once a preservative is present, feed safety is maintained for these preservatives do have their own shelf-lives. An antioxidant might be very effective in controlling rancidity, but its effectiveness decreases with age.

2.3.3.4 Feed processing and handling

Feed processing may cause negative changes that reduce feed quality by encouraging spoilage. Feedmills are avenues for feed contamination. Decker and Zhimin (1998) stated that during certain food processing operations, the balance between the factors, which control oxidative reactions are disrupted and oxidative modification of the chemical components of the tissues occurs resulting in the rapid development of off-flavours. This is because lipolytic enzymes, lipases, are released, which catalyse the hydrolysis of fats to release free fatty acids (Hamilton, 1989).

During some processing operations (grinding, for example), feed particle sizes are reduced, providing a larger surface area for reactions to take place. Grinding also creates friction between grain particles causing the particles to heat up leading to a

significant migration of grain moisture. This encourages mold growth (Herrman, 2002) or increases the rate of fat oxidation. Increase in the rate of fat peroxidation is also accelerated by exposure of feed to oxygen (Sanders, 1985; Sander *et al.*, 1989).

Geoffrey (1982) stated that certain contaminants and additives such as nitrites, nitrates and nicotinic acids together with alkylureas present in meat can lead to the formation of carcinogenic nitrosamides and nicotinates, which have been reported to produce symptoms of gastrointestinal disorders in animals. Nitrites, nitrates and nicotinic acids are used as feed preservatives and to enhance the red colour in meat products. Exposing feed to moisture and high temperatures during processing and storage has been shown to have an adverse effect on quality (Virginia, 1989; Blaha *et al.*, 1990; Valarezo *et al.*, 1997). Lang (1970) suggested that minerals, water-soluble vitamins and proteins may leach out of tissues on heating thus exposing the feed to rancidity and other reactions that result in deterioration.

Nutritionists are most familiar with processing, perhaps with regards to how it greatly determines the rate of feed deterioration. The ways in which products are handled from processing until they are consumed by the animals influence microbial population. Feeds are intended to support life but may be contaminated with pathogenic microorganisms that may endanger the animal's health (Van Schothorst and Brooymans, 1982). It has been noted that processing equipment are major sources of contamination (Jones, 1996; Herrman, 2002). In a study conducted on the fungistatic activity of antifungal compounds in mash and pelleted feed, Paster *et al.* (1985) concluded that pelleting process depressed the antifungal properties of agrosil (a fungistat based on propionic acid).

Bracket (1992) stated that quality and safety of fresh produce depend on their microbiological flora and every step from production to consumption will influence the microbiology of such products. Improper handling and unsanitary equipment used will invariably increase microbial population.

2.3.4 Consequences of contaminated feeds to animals and man

Reviews of related literature have provided information on the consequences of consuming contaminated feeds to both livestock and man. Each aspect of feed spoilage has proven to have detrimental effects and therefore, it is necessary to discuss each based on the type of contamination.

2.3.4.1 Products of rancidity

The unpleasant taste and odour generated from rancidity are undesirable. The effect of FFA level on animal performance has been studied in all major livestock especially poultry (Hamilton, 1989). Rancidity results in loss of quality and acceptability (Galliard, 1989) leading to reduced feed intake. However, consumption of rancid feeds causes both acute and chronic adverse biological effects in animals depending on the level of rancid products consumed (Sanders, 1989). Diarrhoea usually is a common symptom followed by poor growth rate, enlarged liver, fibrosis and anaemia probably caused by the formation of fatty acids with hydroxyl groups from fatty acid hydroperoxides (Sanders, 1985; Shermer and Calabotta, 1985). In addition, the destruction of vitamins A and E by toxic components such as peroxidized fatty acids, oxidized sterols (Sanders, 1989) is a common phenomenon leading to hypo-vitaminoses A and E, respectively (Lin *et al.*, 1989).

Tolerance to rancidity varies from species to species, age and body weight (Bautista, 1992) and also the physiological state of the animal. Polymeric materials may interfere with gut contents and affect vitamin and fat absorption (Sanders, 1989). Cardiomyopathy has been reported in man due to the consumption of rancid feeds (McCollum, 1956). It has been assumed that the feeding of thermally oxidized vegetable oils to experimental animals could lead to hypertrophy of the bile ducts (Kaunitz and Johnson, 1972). Cabel *et al.* (1989) indicated that the presence of subclinical levels of peroxides in feeds could reduce the bird's performance through reduced body weight and poor feed efficiency. Severe oxidation causes a readily detectable illness in broilers called encephalomalacia (Lin *et al.*, 1989).

Research has proven that fat oxidation can cause damage to the membranes, enzymes, vitamins and proteins (Hamilton, 1989). Fat-soluble vitamins are destroyed and sulphur amino acid content is reduced resulting in a reduction in biological value of the feed (Sanders, 1985; Sander *et al.*, 1989) and poor performance. In addition, the biochemical effects of lipid oxidation are the decrease in C_{20-22} polyunsaturated fatty acids in membrane phospholipids, leading to atrophy of the cell membrane (Sanders, 1989). Sanders (1989) also stated that white muscle disease may develop in cattle or sheep with marginal supply of selenium when fed diets with polyunsaturated fats. Muscular dystrophy has been reported in rabbits fed cod-liver oils with vitamin E deficiency (McCollum, 1956), while hypertrophy of the bile has also been reported in experimental animals fed thermally oxidized vegetable oils (Sanders, 1989). However, the consequences are variable depending on the specie of the animal.

2.3.4.2 Mouldy and mycotoxic feeds

Consumption of mouldy and mycotoxic feeds causes the most deleterious effects to both animals and humans (mycotoxicosis and mycosis). Piva and Fabio (1999) defined mycotoxicosis as a syndrome caused by mycotoxin ingestion in animals and man. Mycotoxicosis differs from mycosis according to Ciegler *et al.* (1983) in that, mold growth in the host is not directly involved (it is the toxins that are ingested) in the former, while the later usually involves a generalized invasion of the host by actively growing fungus. Although cases of mycosis in poultry have been reported (Saidi *et al.*, 1994; Johnson *et al.*, 1999; Mody and Warren, 1999), this review will only discuss issues regarding just mycotoxicoses. Biological effects of mycotoxins are many and have been well elaborated by Hinton (1993), D'Mello *et al.* (1999), Placinta *et al.* (1999) and Danicke (2002)

The real understanding of the negative effect of mycotoxins started about 30 – 40 years ago (Hundley, 2001) with the outbreak of "Turkey X" disease in England (Betina, 1989), which encouraged intensive studies on mycotoxins and mycotoxicoses. Physical or apparent effects of mycotoxins range from reduced feed intake and poor feed conversion to a general inability of the animal to thrive (Devegowda *et al.* 1998). Mycotoxins produce a wide range of harmful effects to animals, which could be acute or chronic (Newborne and Butler, 1969; D'Mello *et al.*, 1999) depending on the type of toxin, the dose, animal species (Smith and Moss, 1985; Ciegler, 1989 and Piva and Fabio, 1999) and duration of exposure (Al-azzawi *et al.*, 1978). Young animals are most susceptible to aflatoxin. Piva and Fabio (1999), Newman (2000) and Herrman (2002) indicated that DON, T-2 toxin and zearalenone are known to decrease reproductive

performance in animals. Furthermore, ochratoxin is also reported to cause bone porosity in broilers (Raju and Devegowda, 2000).

In severe cases of mycotoxicosis, death may result (Whitlow *et al.,* 2000). In some cases, there may be feed refusal or decrease in feed intake (Carson and Smith, 1983) in livestock and poultry resulting in serious weight loss (Ciegler *et al.*, 1983; Betina, 1989; Devegowda *et al*, 1998; Raju and Devegowda, 2000; Herrman, 2002;). However, conflicting results have been obtained in experiments addressing whether zearalenone affects growth rate and feed efficiency of young swine (Diekman and Long, 1988). These contradictory viewpoints are not unexpected because, in these experiments using naturally infected feeds containing both zearalenone and DON, it was difficult to determine specific actions of zearalenone alone. In addition, diagnosis of mycotoxicosis is difficult (Nahm, 1995)

Mycotoxin affects the immune system (immunosuppression) by increasing susceptibility to diseases (Pier *et al.*, 1978; Meronuck and Vergel, 1996; Piva and Fabio, 1999; Newman, 2000; Danicke, 2002) especially aflatoxins in poultry (Edds and Bortell, 1983; Huff *et al.*,1992; Nahm, 1995). Aflatoxin consumption increases susceptibility to candidiasis, Marek's disease, coccidiosis and salmonellosis in chickens (Edds *et al.*, 1973; Pier, 1981), pasteurellosis and salmonellosis in turkeys, erysipelas and salmonellosis in pigs, fascioliasis and clostridial infections in calves and intramammary infections in cows as reported by Smith and Moss (1985) and Diekman and Long (1988).

Most toxins cannot be absorbed and detoxified by the liver. Mycotoxins, especially aflatoxins are carcinogenic (Ciegler *et al.*, 1983; Diekman and Long, 1988; Betina, 1989; Huff *et al.*, 1992; D'Mello and Macdonald, 1997; Piva and Fabio, 1999; Whitlow and Hagler, 1999; Whitlow *et al.,* 2000) with the liver and kidney being the most susceptible organs (Devegowda *et al.*, 1998). The hepatotoxic effect of aflatoxin B_1 in laying hens has been reported by Zaghini and Roncada (1998). Aflatoxin causes haemorrhage in tissues (Betina, 1989; Devegowda *et al.*, 1998; Qureshi *et al.*, 1998; Cheeke, 1999; Herrman, 2002). Higher relative organ (liver, kidney, pancreas, heart, proventriculus) weights have been reported in birds fed diets contaminated with aflatoxins (Kubena *et al.*, 1998; Ledoux *et al.*, 1999). In addition, mycotoxins have been reported to interfere with protein synthesis, which results low protein concentration in sera of birds (Kubena *et al.*, 1990; Kubena *et al.*, 1998).

Boysen *et al.* (2000) and Danicke (2002) indicated a relationship between animal health disorder and mycotoxins. Swine are highly susceptible to acute aflatoxin poisoning with severe liver and kidney damage (Cheeke, 1999). At lower concentrations ($\leq$0.4ppm), body weight gain and feed efficiency are reduced. Aflatoxins impair vitamin D_3 absorption (Cheeke, 1999), affect protein, carbohydrate and lipid metabolism (Meronuck and Concibido, 1996) and in severe exposure, cause nausea, vomiting and death (Glahn, 1993). Van den Berghe *et al.* (1990), Parkhurst *et al.* (1992) and Stanley *et al.* (1996) indicated that mycotoxins interfere with metabolism of fats, proteins, vitamins and minerals, which may cause nutritional imbalance. Mycotoxins have been reported to increase lipid peroxidation (Piva and Fabio, 1999). Serum biochemical results show that aflatoxin has been responsible for reducing the blood concentration of glucose, albumin, total protein and globulin (Kubena *et al.*, 1998) as well as bone ash

caused by ochratoxin (Raju and Devegowda, 2000) in chickens. Ochratoxin interfere with bone formation through the destruction of vitamin D_3, which actively takes part in bone mineralisation.

The discovery of aflatoxins emanated from an outbreak of "Turkey X" disease in 1960 which resulted in deaths of thousands of young turkeys, pheasants and ducklings in England (Betina, 1989) from feeds containing aflatoxin-contaminated groundnut meals (Asplin and Carnaghan, 1961; D'Mello and MacDonald, 1997; Smith, 1997; International Hatchery Practice, 1998). After this malaise, in 1960, major developmental studies on mycotoxins and mycotoxicoses were initiated (Meronck and Vergel, 1996).

Even worse, it is not uncommon to find two or more toxins implicated in a particular feed. Mycotoxins in combinations may exert greater negative impact on the health and productivity of livestock and poultry relative to that of individual effects (Devegowda *et al.*, 1998; Danicke,2002). In studies described by Ciegler *et al.* (1983), Huff *et al.* (1986), Smith *et al.* (1997) and D'Mello *et al.* (1999), the presence of two or more toxins expressed synergistic effects that were greater than the sum of the activities of individual agents. In chickens, synergistic, additive and in some cases, antagonistic effects (Huff *et al.*, 1992; Huff and Doer, 1981; Kubena *et al.*, 1990, both cited by Raju and Devegowda, 2000) have been expressed. D'Mello and Macdonald (1997) indicated that high lethality was observed in combinations of toxins whereas the individual mycotoxins had virtually no effect on mortality.

T–2 toxins are found in many cereals, feeds and vegetables (Kubena *et al.*, 1998). Raju and Devegowda (2000) conducted an experiment on broilers exposed to individual and

combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin) with or without esterified-glucomannan. Results showed that antagonistic expressions were exhibited on feed conversion efficiency between aflatoxin and ochratoxin, aflatoxin and T-2 toxin, ochratoxin and T-2 toxin during the first week but profound synergistic effects were expressed during the fourth week. Kubena *et al.* (1998) indicated that aflatoxin and T–2 toxins are important to the poultry industry because of their toxicity and frequency of occurrence in feedstuffs. Loss in weight gain and reduced feed intake due to T–2 toxin contaminations have been reported (Huff *et al.*, 1988; Kubena *et al.*, 1994, both cited by Kubena *et al.*, 1998).

It is worthy to note again that mycotoxin-related problems do not end in reduced feed intake or poor animal performance (Gambrone *et al.*, 1985; Edds and Bortell, 1983, Kubena *et al.*, 1990, cited by Huff *et al.*, 1992; Nahm, 1995; Ledoux *et al.*, 1999) but the toxins are actually transferred into the by-products themselves (Devegowda *et al.*, 1998; Danicke, 2002). D'Mello and Macdonald (1997) cited Anon (1982) and Van Egmond (1989) who reported that aflatoxin M_1 secreted in milk of cows that consumed aflatoxin B_1 contaminated feeds were detected in dairy products destined for human consumption during surveys in several countries including the United Kingdom.

Humans are exposed to mycotoxicoses in three ways. Those that are involved in handling bulk feeds that are contaminated; those involved in laboratory analyses of mycotoxins, and above all, consumers of contaminated feedstuffs or by-products from animals with mycotoxicosis. In mild cases, human consumption of cereals contaminated with tricothecenes causes discomfort of the mouth, throat and stomach followed by inflammation of the intestinal mucosa leading to vomiting and diarrhoea and in severe

cases, bone marrow and haemotopoietic system are damaged, leading to death (Smith and Moss, 1985; Betina, 1989). Many reports from Japan (Palti, 1978; Ciergler *et al.*, 1983; Smith and Moss, 1985) indicate that nausea, vomiting and diarrhoea were associated with the contamination of wheat and rice by *Fusarium spp.* This phenomenon was generally referred to as the "*red-mould disease*". Aspergillosis exists in many diverse forms (Bulmer and Frontling, 1983; Ciegler *et al.*, 1983), all of which are found worldwide in humans.

Ciegler *et al.* (1983) reported that between 1974 and 1975, there was a disease outbreak affecting humans and dogs, with a mortality of about 106 among the 397 cases registered. This effect was due to the consumption of badly moulded corn contaminated with aflatoxin (between 6.5 and 15.6 ppm). Ergotism, one of the oldest food-borne diseases in man caused death of thousands of people in France and other European countries during the Middle Ages (Betina, 1989). Toxin residues may be found in eggs, milk, offals and as such, may represent a potential risk to humans (D'Mello *et al.*, 1999), provided the dosage is lethal.

2.3.4.3 Economic implications

Much of the justification for research and education in mycotoxicology rest in the occurrence of mycotoxicoses as an economic factor in the animal and feed industries (Smith *et al.*, 1976). It is difficult to quantify in economic terms the losses resulting from feed deterioration. Economic losses due to mycotoxins and rancidity in feeds could be determined by assessing the feeds in bulk but the acute, dramatic effect when animals and humans ingest large quantities of toxins and become acutely ill and eventually die cannot be quantified (D'Mello *et al.*, 1999). Practically, the major impact

of mycotoxins and other deleterious substances is yet to be uncovered. This is because the effects of continued low-level consumption of these toxic substances are variable, undramatic and not easily recognised (Betina, 1989).

Information on losses of feedstuffs and feeds is neither adequate nor reliable enough to allow feed scientists to investigate and determine losses in economic terms. However, Ennis *et al.* (1975) estimated that about 30% of crops worldwide are lost due to pest infestations. In addition, CAST (1989) and Devegowda *et al.* (2000) estimated about 25% of the world's cereal grains being contaminated by mycotoxin annually, while Schweigert (1975) reported that food losses from production to consumption in the United States range from 20 – 50%, which in monetary terms, is estimated to be about US $30 billion. Fetuga (1977) argued that feed quality is the major factor implicated in the poor performance and loss of birds in all Nigerian states.

Hinton (1993) identified fungi to be the principal spoilage organisms of feedstuffs (animal food), responsible for reducing the quality of many millions of tons of feed each year worldwide. Paster *et al.* (1985) indicated that fungal growth on grains and feeds can cause severe damage resulting in quality reduction and economic loss. An estimate of about 5% of all harvested grain is lost before consumption (Christensen and Kaufmann, 1974, cited by Lin and Chen, 1995. CAST (1989), cited by Meronuck and Concibido (1996) estimated about 25% of the world's crops are affected annually by mycotoxins, which in monetary terms, translates to billions of US dollars due to loss of crops and animals, part of which is expended on research.

Economic losses associated with exposure to aflatoxin include poor growth and feed conversion, increased mortality, decreased egg production, leg deformity and carcass condemnations in poultry (Qureshi *et al.*, 1998) causing severe economic losses in the industry (Kubena *et al.*, 1998). In the United States, in 1993, an outbreak of Fusarium head blight on barley crop resulted in a loss in yield estimated at 1.6 million metric tons (McMullen *et al.*, 1997, cited by Salas *et al.*, 1999). Similarly, between 1994 and 1998, there was another outbreak causing a significant loss in yield and reduced quality, rendering much of the region's barley seeds unsuitable for malting and brewing due to DON contamination (Steffenson, 1998, cited by Salas *et al.*, 1999). Aflatoxins in many cases may mean the difference between profit and loss to the poultry industry (Jones *et al.*, 1982, Nichols, 1983, Hamilton, 1984, cited by Kubena *et al.*, 1998). The economic impact due to contamination by aflatoxin occurs at all levels of production, marketing and utilisation (Kubena *et al.*, 1990).

2.4 Feed quality control

2.4.1 Aims and definition

Feed quality control is defined by the American Feed Industry Association (AFIA) as all actions directed towards ensuring that the products meet the specifications established by the manufacturer (Jones, 1991). Practically, it means all those measures undertaken to ensure that feed contains all proper nutrients and in right proportions without toxic substances (feed quality). The aim of feed quality control is to ascertain that feeds formulated to meet the nutritional requirements of the animals for which they are produced should be of appreciable quality and safety during feeding (Feltwell and Fox, 1979).

Quality control requires that scientific knowledge be applied from manufacturing to consumption with the optimum level of quality with respect to product conformity, wholesomeness, reliability and safety (Ihekoronye and Ngoddy, 1985). Meyer (2003) stated that the feed that animals eat reflects directly on their health as well as their by-products. In South Africa, policies are in place to achieve food safety, e.g. the Animal Disease Act, 1984 (Act No. 35 of 1984) (NDA, 1988). This law has been enacted to particularly safeguard the health of man and animals.

2.4.2 Concepts of feed quality control

The overall success and profitability of an animal enterprise is greatly determined by quality control in feed production (Richardson, undated). The quality of products obtained in the feed industry involve a number of operations. These are control of raw materials, in-process materials, finished products (Feltwell and Fox, 1979; Richardson, undated) and control of mycotoxins (Jones, 1991; 1996). Raw materials as well as processed feeds are physically examined (which may be by visual, smell or taste appraisals), chemical (e.g. proximate, mycotoxin, rancidity analyses) and biological (use of toxin-binders) assessments are conducted. Whichever method of processing is to be used, requires that it is properly done according to specifications (Jones, 1996). If the tested material is contaminated, it is either properly cleaned or rejected.

2.4.3 Measures adopted to reduce feed contamination and their effects on animal and man

It is important to note that there are other factors other than human forces that determine feed quality assurance as already mentioned. In addition, it is necessary to mention that complete eradication of agents of feed contamination is impracticable (Clark, 2003) but

can be reduced. For quite sometime now, certain food preservative measures have been adopted to reduce food contamination. Such measures include storage of meat at relatively low temperature conditions, meat smoking, haymaking and ensiling, to name a few.

Quality control measures adopted vary according to type of feed, storage equipment available, availability of preservative, cultural background and government policies. There are quite a number of measures undertaken to reduce feed spoilage. These measures are categorised into physical, chemical and biological methods (Goldblatt, 1971; McKenzie *et al.*, 1998; CAST, 1989; Tudor and Petruta, 2001). Physical methods according to van Schothorst and Brooymans (1982) refer to the application of human endeavours e.g. storage at low temperatures, dehydration, separation, dilution, ensiling, fermentation and crushing to reduce spoilage. Chemical methods are those measures taken to reduce spoilage or preserve quality through the use of chemicals such as alkali, organic solvents, acids and gases (D'Mello and MacDonald, 1997). Biological methods include the use of toxin-binders to reduce toxic effects in animals (MacDonald and D'Mello, 1997). The use of living organisms such as cats, dogs (as predators) and nematodes (inhibit fungal growth in the field crops) may be included in this category. For the purpose of this review, priority consideration will be given to prevention of rancidity and mold growth and production of mycotoxins.

2.4.3.1 Mold development and production of mycotoxins

Boysen *et al.* (2000) stated that the problem of mold spoilage is a central issue for feed quality. Efforts have been made to determine cost-effective and safe procedures and product guarantee to effectively tackle decontamination and remediation of

contamination of feedstuffs with mycotoxins (Bailey *et al.*, 1998; Kubena *et al.* 1990; Kubena *et al.* 1998).

Preventive measures in reducing the risk of mycotoxin contamination are of paramount importance (D'Mello and MacDonald, 1997; Placinter *et al.*, 1999) and much more preferable than curative measures (Carter, 2001). The use of fungicides to control fungal diseases and pesticides to control insect pest infestations has been widely exploited. In addition, much attention is being focussed on the use of fungal-resistant varieties of field crops (genetically modified products) that are resistant to fungal colonisation and disease (Brown *et al.*, 1995, Campell and White, 1995, both cited by D'Mello and Macdonald, 1997; Whitlow and Hagler, 1999). As a general rule of thumb, feed storage at low moisture of less than 14 % (Whitlow and Hagler, 1999) and temperature conditions has successfully decreased the proliferation of microbes. The separation of mouldy grains by hand picking resulted in a reduction of 70–90% of aflatoxin and fumonisin in India (Tudor and Petruta, 2001).

Preservative measures using chemicals have been a common practice to reduce mycotoxin production by inhibiting mold growth. Initially, antibiotics such as penicillin, oxytetracycline and ampicilline were used to reduce the toxic effects of mycotoxins on animals. This then gave way to mycotoxin-binders because it was generally agreed that the use of antibiotics in agriculture leads to an increase in the incidence of resistant bacteria in the intestines of animals (WHO Report, 1997; Witte, 1998, both cited by Salyers, 1999). Propionic, sorbic and phosphoric acids as well as sulphur dioxide and related products as feed preservatives have been widely used with positive results in preventing mold growth and development. These products have proven to be effective

(Paster *et al.*, 1985; Gibson *et al.*, 1988; Higgins and Brinkhaus, 1999, Pelhate, 1973, both cited by Carter, 2001)

Devegowda *et al.* (2000) was of the opinion that the approach to problems associated with mycotoxin contamination should go beyond a simple focus on fungal growth inhibition, to include an evaluation of poultry performance. Biological decontamination methods are those measures undertaken to minimize the effect of toxins on the animals by modifying the diet and improving on the nutritional content. An example is inclusion of vitamin C (Netke *et al.*, 1997, cited by Piva and Fabio, 1999; Tudor and Petruta, 2001) to promote growth by stimulating the immune system through enhanced white blood cell function and utilization of lysine and methionine. Mycotoxin binders such as mannan-oligosaccharides (derived from the cell wall of yeast called *Saccharomyces cerevisiae*) are also used to reduce toxin absorption in the gastrointestinal tract (Shane, 1999; Devegowda *et al.*, 2000). In addition, mineral-related substances such as hydrated sodium calcium aluminosilicate (HSCAS) acting similarly to mannan-oligosaccharides, have been shown to form highly stable complexes with aflatoxins in poultry and pigs (Devegowda *et al.*, 2000; Carter, 2001). These complexes so produced are excreted via the anal passage.

2.4.3.2 Rancidity

The most important aspect of preventing the occurrence of rancidity is the use of antioxidants. An antioxidant is defined as any substance, which is capable of delaying, retarding or preventing the development in food of rancidity or other flavour deterioration due to peroxidation of fats (Coppen, 1989). It is important to note that

antioxidants prevent oxidative rancidity but not hydrolytic rancidity from taking place in feeds.

It is a paradox that rancidity cannot take place in living materials (biological membranes) as stated by Sanders (1989). This is because lipids are unlikely to react with air or are catalysed due to the balance between factors that control oxidative reactions, which are disrupted at processing (Decker and Zhimin, 1998). Furthermore, plant cells contain natural antioxidants (vitamin E) and enzymes superoxide dismutase that help prevent peroxidation during storage. Unfortunately, most of these antioxidants are themselves unstable, i.e. destroyed by peroxides. In addition, little has been done to alleviate the effects of rancid feeds on animal performance. Once a feed is rancid, it is rancid. Paradox

All actions geared towards preventing the occurrence of rancidity are important. First of all, measures adopted to prevent rancidity start from the feed factory. Before attempting to adopt measures to prevent the occurrence of rancidity in feeds, it is imperative to have a clear understanding of the various factors that influence rancidity and tackle them individually. The use of antioxidants becomes necessary if feeds are to be stored at adverse conditions. Decker and Zhimin (1998) indicated that in preventing oxidative deterioration, it is required that the pro-oxidative effects of processing techniques are minimised and concentrations of substrates (oxygen and fatty acids) are altered. This requires the utilization of antioxidants.

Fats should not be included in diets by more than 10% (Njobeh, 1997) or unstable fats or other pro-oxidants in feeds should be minimised as much as possible (Rumsey,

1978). Coconut and palm kernel oils are usually preferred because the rate of oxidation of these products is low (Berger, 1989). Feed preservation at a moisture content of 5% has been shown to reduce the rate of hydrolytic rancidity (Galliard, 1989; Njobeh, 1997).

Prevention of mold growth and insect infestation in feeds has been used as a measure to reduce rancidity in feeds. This is because the presence of these organisms helps to increase the temperature within the feeds. A 10% increase in temperature doubles the rate of oxidation (Berger, 1989). Low temperature and low relative humidity during storage are beneficial in maintaining feed quality. Since oxidative rancidity cannot take place in the absence of oxygen (Berger, 1989), the product can be stored in low oxygen environment to minimise spoilage. Certain means of fending off oxidation are vacuum packaging, storage at freezing point and packing under an inert gas to exclude oxygen. But these measures are not practical (Coppen, 1989) with feeds because of their bulkiness, nor attempt to completely exclude oxygen from an environment.

Although there are over a hundred chemicals tested to determine their effectiveness in preventing oxidation, only a few of them have proven to be effective. The use of antioxidants in the US dates as far back as to the 1880s (Rumsey, 1978). Antioxidants must have the following characteristics: effective in preserving quality at low concentrations, non-toxic to animal and man and economical (Rumsey, 1978). Ethoxyquin (Cabel *et al.*, 1988), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Rumsey, 1978; Gordon and Machlin, 1959; Williams *et al.*, 1960, both cited by Lin *et al.*, 1989; Bautista *et al.*, 1992) have been found to be very effective synthetic antioxidants.

The tocopherols (vitamin E) have also been used in preventing oxidation in broiler diets (Waldroup *et al.*, 1988) and poultry meat (Marusich *et al.*, 1974). Ascorbic acid (Vitamin C) is also a natural antioxidant alongside vitamin E (Cheeke, 1999). Vitamin E reacts with cell membranes and other lipid environment thereby preventing PUFA from peroxidation (Bramley *et al.,* 2000). Bramley *et al.* (2000) also indicated that intensive research on vitamin E stemmed from the hypothesis that its ability to prevent free radical-mediated tissue damage may play a role in stimulating the immune system.

In most situations, two or more antioxidants are used in combination to provide a synergistic effect in preventing oxidative rancidity in feeds. Resultant effects are much better than for individual antioxidants. The mechanical pathways of antioxidant reaction with free radical formed from dehydrogenation of fats to prevent oxidation are fully described by Rumsey (1978).

The obvious measure to implement if all the above precautionary steps taken to combat rancidity have failed is to avoid the use of feeds that may have high levels of free fatty acids or peroxide value. However, other schools of thought suggest that mixing rancid feeds with fresh (devoid of rancidity products) feed to reduce the concentration of peroxide or free fatty acid levels is another precautionary step to reduce their toxic effects to animals. It can be suggested that supplementing rancid feeds with fat-soluble vitamins and amino acids prior to feeding could alleviate the possible effects of rancid feeds on animals. This could be a possibility considering the fact that oxidative rancidity results in the destruction of these nutrients and also reduces the energy value of the feed (Rumsey, 1978). It was not until 1957 that the role of selenium in preventing

liver necrosis in rats and exudative diathesis in chicks was identified (Maiorino, 1992). In addition to selenium, ascorbic acid and vitamin E have been shown to prevent adverse effect of rancid feed in animal tissues (Maiorino, 1992; Cheeke, 1999; Piva and Fabio, 1999).

2.4.3.3 Introduction of HACCP

HACCP (Hazard Analyses Critical Control Point) was introduced about 30 years ago by the United States Food and Drug Administration (FDA, 2001). Its introduction was to prevent hazards in seafood and juice for astronauts but is now applicable to all aspects of animal product related industries. It is defined according to MacGillivray (2001) as a concept that systematically identifies, evaluates and controls (potential) hazards that are significant for food safety. This program was designed to focus on preventing hazards (biological, chemical or physical agents) that could cause food-borne illnesses by the application of scientific-based controls, from raw materials to finished products (MacGillivray, 2001).

Quality assurance system alongside some organisations like the United States Department of Agriculture (USDA), European Union (EU), European Feed Manufacturers Federation (FEFAC) and Animal Feed Manufacturers Association of South Africa (AFMA) enforce good manufacturing practices for quality assurance in the animal production sector.

2.5 Conclusion

The review has attempted to discuss all areas that concern feed quality. A few specific aspects of feed quality may not have been mentioned or discussed in order to minimize the volume of the thesis and keep within the objectives of the project.

CHAPTER THREE

3.0 Research Outline and Methodologies

3.1 Introduction

Animal feeds are naturally liable to contamination from the time they are at an ingredient level, to the time they pass through processing and storage, until they are ingested by animals. The consequences associated with feed contamination range from loss in nutrients to production of toxins, which have detrimental effects to both animals and humans. Climatic conditions and to a lesser extent, man-made factors are responsible for feed contamination which causes severe losses to the animal industry in particular, and to the consumer in general.

Studies on the causes and effects of feed contamination as well as measures adopted to reduce feed spoilage have been widely exploited in all species of animals and in diverse fields of animal science. Provision of a suitable environment for feed storage for the tropical and sub-tropical regions and human measures adopted to reduce feed spoilage is the main objective of this research.

3.2 Protocols

To meet the objectives of the present study, each study was conducted in two distinct phases. In experiment one, the first phase involved an assessment of the impact of storage conditions (temperature and relative humidity) and additives on feed quality during storage. While in the second phase, a feeding trial was conducted to determine the natural pattern of mycotoxicosis in relation to whole body growth, internal organs and serum composition. To evaluate the influence of fat level on feed quality,

experiment two was conducted similarly to experiment one but with high fat diet. Both experiments were run concurrently.

3.3 Experimental diets

3.3.1 Experiment one

This experiment was conducted with a commercial broiler starter diets obtained from a commercial feed producing company-Epol, Pietermaritzburg, Kwazulu-Natal, South Africa. The feed was formulated to meet the requirements of broiler chicks as specified by National Research Council (NRC) (1994). To meet the objectives of this study, the diet (A) was compounded to contain low fat content. The main objectives were to determine the degree of spoilage over a prescribed storage period of two months, evaluate the extent to which preservatives (mold inhibitor and antioxidant) would enhance feed quality, and assess the effects of contaminated feeds and mycotoxin-binder on the performance of broiler chicks. The materials were received on the same day of production. The composition of the diet is shown in Table 3.1.

Table 3.1 Composition of feed A.

Ingredient (g/kg)	Diet
Yellow maize	615.3
Soya Hi pro(46%)	231.5
Sunflower oilcake	51.0
Fishmeal (Chilean)	72.5
Limestone powder	13.0
Monocalcium phosphate	9.7
Salt (NaCl)	1.7
Molasses	2.3
Choline chloride	0.7
Premix (Starter)	0.2
Cycostat (Robenidine)	0.5
Zinc bacitracin	0.4

3.3.1.2 Chemical composition

Prior to storage, i.e., when the diet was still fresh (day 0), samples were collected, put in sealed plastic bottles and subjected to proximate analyses. These analyses were conducted by the technical personnel at the Animal and Poultry Science Department, University of Natal, Pietermaritzburg, South Africa.

Protein and amino acids were measured by the method described by AOAC (2000). Crude protein (nitrogen content x 6.25) of the diets was determined on a LECO FP-2000 Nitrogen Analyser, Saint Joseph, MI, USA (Appendix I). Amino acids (methionine and lysine) were analysed by the photometric ninhydrin method (Moore and Stein, 1984) on a Beckman amino acid analyser (Beckman Instruments, Inc., Spinco Division, Palo Alto, USA). Dry matter was determined according to AOAC (2000). Fat was extracted from the sample with petroleum ether using the Soxhlet procedure (AOAC, 2000). Crude fibre analysis (Appendix I) was determined according to the materials and procedures of AOAC (2000). Determination of ash in feed was done according to AOAC (2000).

Calcium and phosphorus were determined by AOAC (2000) using a Varian Spectra AA-200 Atomic Absorption Spectrophotometer (Varian Inc., Palo, Alto, C.A., USA) and a Technicon Autoanalyser II (Technicon Instruments, NY, USA), respectively. Materials and procedures for Ca and P are presented in Appendix I. Nitrogen corrected apparent metabolizable energy (AME_n) and true metabolizable energy (TME_n) of the diets were determined according to the method described by Sibbald (1986) in Appendix I. Iron, copper and zinc were determined by atomic absorption spectrometric methods according to AOAC (2000) as shown in Appendix I.

3.3.1.3 Rancidity test

Sub-samples were obtained from the diets with the use of a sampler (18cm long and 1cm in diameter) from four different parts of the bag, mixed thoroughly, put in sealed plastic bottles and then stored at a temperature of –15 °C for rancidity analyses at one month interval (x3). Determination of peroxide value (Pv) and free fatty acids (FFA) according to AOAC (1990) were carried out by titration method with the assistance of personnel at the Animal and Poultry Science Laboratory, University Of Natal, Pietermaritzburg, South Africa. The procedures for the extraction of fats from feeds and determinations of FFA and Pv have been described in Appendix IV.

3.3.1.4 Feed microbiology

Sub-samples for mycotoxin analyses were obtained from the top, middle and bottom of the bags with the aid of a sampler and put into sealed plastic bottles, placed in coolers maintained at very low temperature with the aid of ice bags and taken to the Human Physiology Department, University of Natal, Durban, South Africa, where they were sub-cultured for fungal identification and screened for 20 mycotoxins using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), according to Dutton (1993) with the assistance of the laboratory personnel. Materials and procedures for fungal identification and determination of levels of toxin production are described in Appendix III.

Mycotoxin identification

The toxins tested were aflatoxins, citrinin, kojic acid, ochratoxin, patulin, penicillic acid, sterigmatocystin, trichothecens, zearalenone and moniliformin. The multi-

mycotoxin screen technique was used which consists of a flow pattern of sampling, separation, extraction and confirmation of results as described in Appendix III according to Dutton (1993).

3.3.2 Experiment two

This experiment was conducted with a commercial broiler starter diets obtained from a commercial feed company-Epol, Pietermaritzburg, South Africa. The feed was formulated to meet the requirements of broiler chicks as specified by National Research Council (NRC) (1994). To meet the objectives of this study, the diet (B) was compounded to contain high fat content. The main objective was to determine the influence of high fat level on feed quality over a prescribed storage period of two months, evaluate the extent to which preservatives (mold inhibitor and antioxidant) would enhance feed quality, and assess the effects of contaminated feeds and mycotoxin-binder on the performance of broiler chicks. The materials were received on the same day of production. The composition of the diet is shown in Table 3.3.

3.3.2.2 Chemical composition

Prior to storage, i.e., when the diet was still fresh (day 0), samples were collected, put in sealed plastic bottles and subjected to proximate analyses.

3.4 Ethical clearance for animal management

The studies reported in this thesis were authorized by the Animal Ethics Committee, University of Natal, Pietermaritzburg, South Africa. Experimental materials and procedures were designed to minimize stress and pain to the birds. Only diets that could be practically used in the broiler chicken enterprise were formulated and assessed. Deliberate act of feed contamination with fungi prior to storage was duly

acknowledged. Prior to the collection of internal organ samples, chicks were euthanatized by asphyxiation with carbon dioxide.

Table 3.3 Composition of feed B

Ingredient (g/kg)	Diet
Yellow maize	556.2
Wheat middling	50.0
Soya Hi pro(46%)	250.0
Fishmeal (Peruvian)	16.0
Fishmeal (Chilean)	20.0
Poultry byproduct	30.0
Bone meal	48.5
Blended oil	10.0
Salt (NaCl)	2.6
Molasses	9.0
Methionine	3.1
Choline chloride	0.7
Lysine HCl	1.2
Premix (Starter)	1.5
Threonine	0.3
Cycostat (Robenidine)	0.5
Zinc bacitracin	0.4

3.5 Preparation of inoculants

Ten mg of sodium dodecasulphate (SDS) was dissolved in 100 ml of distilled water. Ten ml of the dissolved solution was transferred into each McCartney bottle, and autoclaved for 1 hr 30 minutes. The bottles were then allowed to cool to a temperature of 25 °C. Spores of *Fusarium moniliformes* and *Aspergillus paraciticus* (common feed contaminating fungi) were individually obtained from a culture medium with the aid of a glass rod and separately put into each McCartney bottle containing the dissolved solution and shaken vigorously. The fungal spores were obtained from Human Physiology Department, University Of Natal, Durban, South Africa.

3.5.1 Diet inoculation and inclusion of a preservative mixture

Prior to storage, the diets were spiked with a solution containing spores of *F. moniliformes* and *A. paraciticus* to encourage fungal growth and subsequent toxin production in the diets. These are common feed contaminating fungi. There was a control, not spiked, for microbial analyses.

A preservative mixture composed of a mould inhibitor (Mold Zab) and an antioxidant (Banox E) (at a ratio of 1:3 i.e. 250 g/750 g per tonne of feed) obtained from Alltech Inc. was thoroughly mixed with one of the two portions of the diets according to the manufacturer's recommendation (Appendix II). Forty ml of SDS solution was thoroughly mixed with each of the experimental diets. This inclusion of preservative mixture was to determine the extent to which a mold inhibitor and antioxidant prevent mold growth and reduce the rate of oxidation of fats, respectively in the diets.

3.6 Phase I: Feed storage and analysis

Temperature (15 and 30 °C) and relative humidity (50 and 80%) was used during storage of feeds. The feeds were stored in 50 kg bags placed adjacent to one another (1m apart) on wooden bases (10cm high). Four environment-controlled chambers at Ukulinga Research Farm, Department of Animal and Poultry Science, University Of Natal, Pietermaritzburg, South Africa, were used for storage. The dimension of each chamber was 12 x 4 x 8 m. Chambers 1 and 2 had the same temperature 15 °C but differed in the relative humidity of 50% and 80%, respectively. Chambers 3 and 4 had the same temperature of 30 °C but different relative humidities of 50% and 80%, respectively. The description of the four storage conditions is presented as follows:

- Chamber 1 (15 °C temperature and 50% relative humidity)

- ❖ Chamber 2 (15 °C temperature and 80% relative humidity)
- ❖ Chamber 3 (30 °C temperature and 50% relative humidity)
- ❖ Chamber 4 (30 °C temperature and 80% relative humidity).

3.6.1 Environmental conditions

The environmental conditions were adjusted to meet the requirements of the research. Room temperature and relative humidity were monitored and adjustments made on controlled heaters and humidifiers where necessary 10 days prior to the commencement of storage. Data on daily temperature and relative humidity readings were recorded from a hygro-thermometer suspended just above the bags throughout the storage period. Daily readings and recordings were carried out between 09:00 and 11:00 and are presented in Figure 3.1. Data on daily temperature were similar to the intended conditions (standard deviation of between 0.30 to 0.63). In regards to relative humidity, there was a disparity in the readings to the intended condition but there was however, the difference between high and low RH storage conditions (standard deviation of between 3.25 to 5.65), which did not have any effect on, the parameters measured.

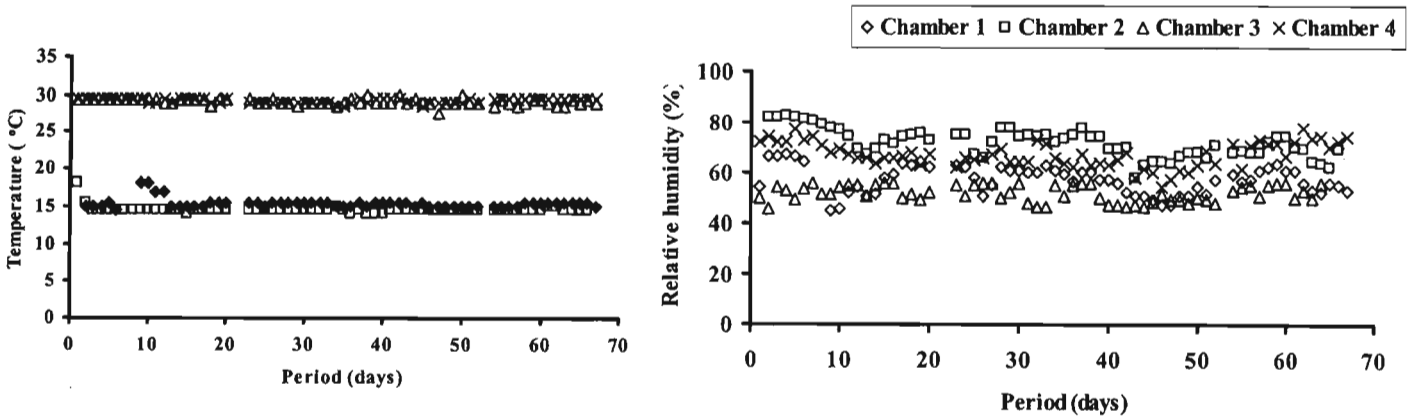

Figure 3.1 Graph of daily temperature (*left*) and relative humidity (*right*) readings against storage period.

During the two-month period of storage, sub-samples were also collected at one-month interval (1 and 2 months of storage) with the use of a sampler sterilized with 75% ethanol so as to prevent cross contamination of samples by fungi (recontamination). The sub-samples obtained were used to determine chemical compositions (crude protein, calcium, phosphorus, ash, moisture content, iron, zinc, and copper) as described in Appendix I. Free fatty acids and peroxide value (Appendix IV) were also determined. In addition, microbial assessments (Appendix III) were conducted.

3.6.2 Post-storage treatment

At the end of the storage period, a detoxifier, MTB 100, an organic product derived from cell wall of Saccharomyces yeast obtained from Alltech Inc., Nicholasville, KY, USA, was thoroughly mixed in one of the two portions of the stored feeds at a rate of 2 kg/ton of feed according to the manufacturer's recommendation (Appendix II). The purpose of inclusion of the detoxifier was to prevent effects of mycotoxins in animals.

3.7 Phase II: Feeding trial

A 2 x 2 x 2 x 2 factorial design was used in which a commercial broiler starter diet with or without preservative mixture and with or without a detoxifier were stored in each of the four environment-controlled chambers as described in section 3.6. These combinations yielded 16 main treatments.

3.8 Animal management and sample collection

Four hundred and eighty day-old broiler chicks (Ross) were obtained from a commercial hatchery (National Chicks, SA Pty). Three replicate pens of male, female

and male/female (1:1) each containing ten chicks, were randomly assigned to each treatment and fed from day-old to twenty-one days of age.

The chicks were reared in single-tier electrically heated battery brooder cages (pens). The chicks were weighed at the start of the trial and randomly assigned to the experimental diets (mash form) and fed *ad libitum* until 21 days. Drinking water was provided *ad libitum* from two nipple drinkers per cage. Artificial lighting from normal fluorescent tubes was provided on a 24 hr basis throughout the study. Feed intake and body weight were measured at the end of each week. Mortality was recorded as it occurred. Room temperature was maintained in accordance with the requirements for chicks between day-old and 21 days.

3.8.1 Measurements

The measures of response were feed intake (g/bird), body weight (g/bird), feed conversion efficiency (FCE)(g gain/kg feed intake), % tibia ash, relative visceral organs weight (g/100g body weight) as well as total phosphorus (mmol/l), serum protein, albumin and globulin (g/100ml) in serum samples. Growth parameters are influenced by feed quality. Furthermore, visceral organs (target site for mycotoxins) and serum biochemistry have been used as diagnostic tools in assessing the influence of occurrence of mycotoxicosis in birds (Bailey *et al.*, 1998; Kubena *et al.*, 1998) with liver and kidney being the organs of attention. Bone ash was measured with the assumption that mycotoxins (ochratoxin) interfere with vitamin D_3 metabolism, which plays a prominent role in bone mineralization (Huff *et al.*, 1977, cited by Raju and Devegowda, 2000).

Feed intake (FI) per week per bird was calculated as the difference between the feed-in and feed–out, all divided by the sum total of the birds present within the cage per day within the week the feed was consumed. The total FI is obtained by summation of the weekly FI. Body weight was calculated as the average weight per cage. Feed conversion efficiency was determined as the average weight gain divided by total feed intake.

At the end of the trial, four birds were randomly selected per pen and killed by carbon dioxide asphyxiation. From each pen, a bird was bled by cardiac puncture and blood sample was obtained, put in a test tube and processed by centrifuge. The serum was obtained, put in a sealed mailing tube, snap-frozen in liquid nitrogen and immediately sent to Allerton Laboratory (Pietermaritzburg, South Africa) for determination of total phosphorus, serum protein, albumin and globulin. The time taken from sacrifice to collection of serum was 10 – 15 min.

3.8.2 Haematology

Serum biochemistry was analysed using UV-VIS spectrophotometer according to Heine *et al.* (1989) modified by Biochemistry Laboratory, Allerton PVL, Pietermaritzburg, South Africa. Details are provided in Appendix V.

3.8.3 Organ morphology

The birds were dissected and visceral organs, viz: liver, gizzard, spleen, pancreas and proventriculus were carefully removed, cleaned of excess fats and weighed.

3.8.4 Tibia ash

The right legs of the four sacrificed birds were then collected in sealed plastic bags and then sent to the Animal and Poultry Science Departmental Laboratory, University of

Natal, Pietermaritzburg, South Africa for tibia ash analysis. The tibias from four birds per cage were obtained by boiling in distilled water for 5 min. The muscle and cartilage were then scrapped and discarded. These were then pooled and stored in sealed plastic bags at 0 °C until they were analysed for ash as previously described.

3.9 Data analysis

The experimental structure was a 2 x 2 x 2 x 2 factorial and data obtained in this study were analysed by the general linear model (GLM) of Minitab. Unlike in the rancidity analyses, some of the high order interactions in the model were eliminated in order for the model to be "full rank" (Minitab, 1998). This was done because there were too many factors (4) in which some degree of freedoms might be lost during analysis. It was assumed the interactions eliminated were as important as the major factors retained. A one-way analysis of variance (ANOVA) was used to derive mean values, which were then compared by the least significant difference. Mean values were deemed to be different if level of probability was ≤ 0.05.

CHAPTER FOUR

4.0 Effects of storage conditions and supplements on nutrient composition and nutritive value of maize-based diets for broiler chickens (Experiment One).

4.1 Results

4.1.1 Chemical composition of the diets

The nutrient composition of the diet prior to storage is shown in Table 4.1 while Fig. 4.1 presents laboratory results of the nutrient composition of the diets throughout the experimental storage period of two months. The dry matter content of the diets after one month of storage was higher than that of the fresh diets, regardless of storage temperature and RH. On the contrary, DM content of most of the diets had decreased by 1.6% after two months of storage. After two months of storage, CP content substantially increased except in diets stored at low temperature and low RH. After one month of storage, fat content of diets with inhibitor was maintained regardless of storage temperature and RH, while diets without inhibitor were found to have increased in fat content. Increments in fat content were observed with further storage, irrespective of treatment (Fig. 4.1).

Table 4.1 Nutrient compositions (g/kg) of the fresh diets.

Nutrient	Composition
Dry matter	886.0
Crude protein	193.0
Fat	34.0
Crude fibre	36.0
Calcium	12.0
Phosphorus	7.0
Methionine	3.0
Lysine	10.0
AME_n (MJ/kg)	12.6
TME_n (MJ/kg)	13.0
Iron (mg/kg)	280.0
Zinc (mg/kg)	130.0
Copper (mg/kg)	20.0

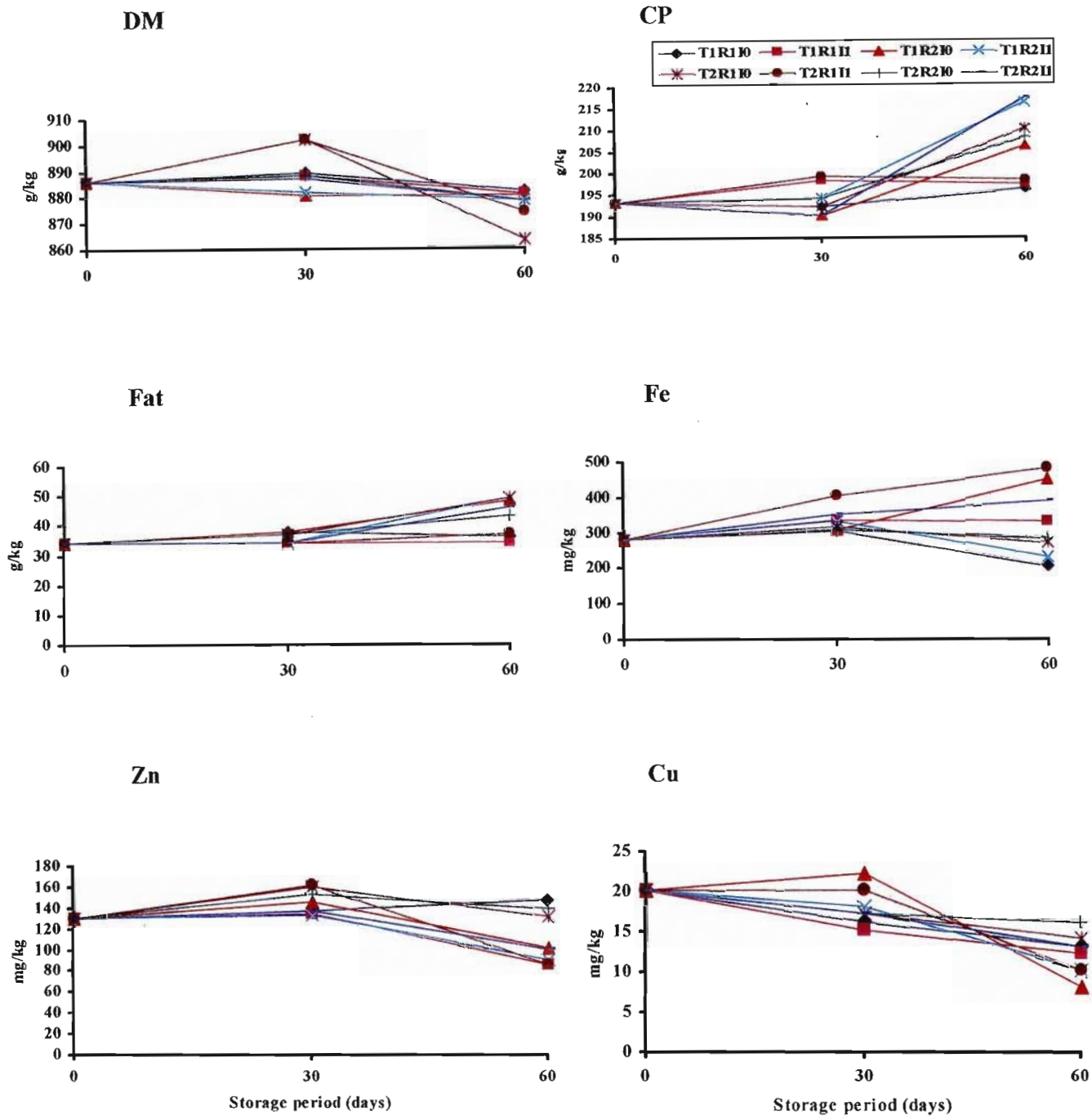

Figure. 4.1. Changes in the nutrient composition of the diets over time. T1, 15°C; T2, 30°C; R1, RH 50%; R2, RH 80%; I0, without inhibitor; I1, with inhibitor. Samples were analysed in duplicates and mean values derived. No statistical analysis was performed.

The changes in micro-mineral content during storage are also shown in Fig. 4.1. Results indicate that there was an increase in the concentrations of Zn and Fe by about 7% after one month of storage but the reverse was the case for Cu. During this period, Zn content was observed to increase in diets without inhibitor. In the second month, there was no trend in changes in the concentration of Fe, i.e. some had further increase while others decreased with storage. It was also observed after two months of storage that there was a sharp drop in the concentration of Zn (180 to about 150mg/kg feed) in the diets regardless of storage temperature and RH. The concentration of Cu in the diets decreased throughout storage and this was much more noticeable after two months of storage.

4.1.2 Feed microbiology

Microbiological analyses of feed samples prior to storage showed some fungi. The species that were detected include *Aspergillus flavus*, *A. parasiticus*, *Penicillium spp* and *Fusarium moniliformes*. No toxins were, however, detected.

4.1.3 Rancidity

All the diets regardless of storage temperature and relative humidity were subject to lipid hydrolysis. The free fatty acid contents of the diets increased ($p<0.001$, $R^2= 0.73$) from an initial value of 3.5 to an average of about 12% over the experimental period of 60 days (Table 4.2 and Appendix VI). Storage of diets at high temperature significantly ($p<0.001$) increased FFA content to 14% as shown in Table 4.2. Increase in the concentration of free fatty acids of the diets after two months of storage follows a similar trend to that after one month (Appendix IV). At low temperature, the rate of fat hydrolysis was much slower than at high temperature. FFA content of the diets was increased ($p<0.001$) by high temperature in low RH environment. Similarly, high

Table 4.2. Effects of varying feed storage conditions and antioxidant inclusion on The concentration of free fatty acids (%) and peroxides (mEq O_2/kg fat).

Temp	RH	Inhibitor	Period	FFA	PV
15	50	-	1	3.5^{j}	0.6^{i}
			2	6.5^{h}	3.1^{f}
			3	8.6^{f}	5.7^{c}
		+	1	3.5^{j}	0.6^{i}
			2	5.7^{i}	1.6^{h}
			3	9.3^{ef}	4.2^{e}
15	80	-	1	3.5^{j}	0.6^{i}
			2	7.6^{g}	3.5^{f}
			3	10.9^{d}	6.0^{c}
		+	1	3.5^{j}	0.6^{i}
			2	6.7^{h}	2.5^{g}
			3	11.8^{c}	5.0^{d}
30	50	-	1	3.5^{j}	0.6^{i}
			2	10.0^{ef}	4.4^{de}
			3	$8.0f^{g}$	4.1^{e}
		+	1	3.5^{i}	0.6^{i}
			2	10.4^{de}	2.6^{fg}
			3	12.9^{b}	6.3^{bc}
30	80	-	1	3.5^{j}	0.6^{i}
			2	10.4^{de}	4.8^{d}
			3	15.7^{a}	8.2^{a}
		+	1	3.5^{j}	0.6^{i}
			2	10.0^{de}	3.1^{f}
			3	16.5^{a}	6.6^{b}
	SEM			0.23	0.13
		Source of variation			
Temp				***	***
RH				***	***
Inhibitor				***	***
Period				***	***
Temp*RH				***	***
Temp*Inhibitor				***	***
Temp*Period				***	***
RH*Inhibitor				***	***
RH*Period				***	***
Inhibitor*Period				***	***
Temp*RH*Inhibitor				***	***
Temp*RH*Period				***	***
Temp*Inhibitor*Period				***	***
RH*Inhibitor*Period				***	***
Temp*RH*Inhibitor*Period				***	***

^{a,b,c,d,e,f,g,h,I,j} Mean values in the same column not sharing a superscript are significantly different
***** p<0.001 NS: not significant.**
SEM is the standard error of difference between the mean values.

RH at low temperature also significantly ($p<0.001$) increased FFA level. Inclusion of an inhibitor in the diets did not decrease the concentration of FFA over two months of storage (Table 4.2). The highest concentrations of FFA (15.5 – 16.5%) were observed in diets stored at high temperature with or without inhibitor.

Various levels of interactions observed between the four factors were significant ($p<0.001$). Peroxide value of the diets follow a similar trend observed for free fatty acid concentrations except for the fact that the Pv of the feeds was significantly decreased ($p<0.001$) by the inhibitor irrespective of storage temperature and RH as shown in Table 4.2. The presence of the inhibitor reduced the concentration of peroxides in the diet by about 10% over the entire storage period. The Pv of the diets rapidly increased ($p<0.001$, $R^2 = 0.84$) from an initial value of 0.6 to an average of about 6 mEq/kg fat over the experimental period of 60 days (Table 4.2 and Appendix VI). Diets that were stored at high temperature had significantly ($p<0.001$) increased Pv from 0.6 to 8.0 mEq O_2/kg fat.

Increase in the Pv of diets after two months of storage followed a similar trend to that after one month (Table 4.2). The Pv of the feeds was increased ($p<0.001$) by high temperature in low RH environment. Similarly, high RH at low temperature also significantly ($p<0.001$) increased the concentration of peroxides. Inclusion of an antioxidant in the feeds irrespective of storage temperature and RH decreased ($P<0.001$) the Pv. In general, all interactions between the four factors significantly affected ($p<0.001$) the Pv.

4.1.4 Production parameters

The effects of temperature, relative humidity, inhibitor and mycotoxin-binder on the performance of broiler chicks are presented in Table 4.3. Birds fed diets stored at low RH had significantly ($p<0.01$) higher body weights. This was much more evident in diets that were stored at high temperature condition. However, storage temperature did not significantly affect body weight of birds. It was also observed that inclusion of a detoxifier in diets significantly improved ($p<0.001$) body weight (Table 4.3). Inclusion of the inhibitor in the diet failed to influence body weight of birds. The FCE of birds fed diets stored at low RH was significantly higher ($p<0.001$) than that at high RH. This effect was much more noticeable at high storage temperature where there was a large depression in FCE.

Similarly, birds that consumed diets stored at low temperature had higher ($p<0.05$) FCE than those that were placed on diets stored at high storage temperature. Unlike the inhibitor, the presence of a detoxifier in diets significantly ($p<0.01$) improved FCE of birds, regardless of storage temperature and RH. The FCE of birds fed diets with a detoxifier was higher than that of birds on diets without detoxifier.

In contrast to body weight and feed conversion efficiency, feed intake was not significantly affected by any of the factors. Among the interactions between factors tested, temperature x inhibitor was found to significantly ($p<0.05$) influence feed intake. In addition, interaction between temperature and relative humidity influenced ($p<0.01$) body weight (Table 4.3). No other interactions affected ($p<0.05$) any of the production parameters mentioned.

Table 4.3. The effect of varying storage temperature, relative humidity, inhibitor/antioxidant and detoxifier on feed intake (g/bird), final body weight (g) and feed conversion efficiency (g weight gain/kg feed).

Temp (°C)	RH (%)	Inhib.	Detox.	Feed intake	Body weight	FCE
15	50	-	-	926^{def}	620^{b}	608^{abcd}
		-	+	963^{abcde}	654^{ab}	622^{abc}
		+	-	906^{f}	626^{ab}	630^{ab}
		+	+	934^{cdef}	658^{ab}	646^{a}
15	80	-	-	974^{abcd}	626^{ab}	588^{abcd}
		-	+	979^{abc}	656^{ab}	615^{abc}
		+	-	955^{abcde}	617^{b}	588^{abcd}
		+	+	922^{def}	645^{ab}	640^{ab}
30	50	-	-	917^{ef}	631^{b}	628^{ab}
		-	+	951^{abcde}	646^{ab}	621^{abc}
		+	-	941^{bcdef}	638^{ab}	621^{abc}
		+	+	988^{a}	675^{a}	628^{ab}
30	80	-	-	920^{def}	554^{c}	543^{d}
		-	+	947^{abcdef}	607^{b}	583^{bcd}
		+	-	947^{abcdef}	586^{bc}	562^{cd}
		+	+	982^{ab}	657^{ab}	612^{abc}
			SEM	43.07	19.35	21.91
Source of variation						
Temperature				NS	NS	*
Relative humidity				NS	**	***
Inhibitor				NS	NS	NS
Detoxifier				NS	***	**
Temp x RH				NS	**	NS
Temp x Inhibitor				*	*	NS
RH x Inhibitor				NS	NS	NS
Temp x RH x Inhibitor				NS	NS	NS

[a,b,c,d,e,f] Mean values in the same column not sharing a superscript are significantly different p<0.05, ** p<0.01 & *** p<0.001 NS not significant.
SEM is the standard error of the means.

4.1.5 Mortality

There was no pattern in the mortality recorded. Total mortality was less than 6%. Treatment did not in any way affect mortality record throughout the experimental period.

4.1.6 Visceral organ weight

The effects of temperature, relative humidity, inhibitor and detoxifier on the relative visceral organ weight of the broiler chicks are presented in Table 4.4.

Table 4.4 Effect of varying storage temperature (temp) (°C), relative humidity (RH) (%), inhibitor/antioxidant and detoxifier on the relative weight (g/100g body weight) of visceral organs.

Temp	RH	Inhib.	Detox.	Liver	Heart	Prov.	Giz.	Spleen
15	50	-	-	4.24^{b}	0.83^{ab}	0.69^{ab}	4.62^{a}	0.14^{abc}
		-	+	3.63^{c}	0.78^{ab}	0.65^{ab}	3.40^{c}	0.13^{abc}
		+	-	3.50^{cd}	0.76^{b}	0.61^{ab}	3.86^{bc}	0.11^{bc}
		+	+	3.30^{cd}	0.70^{b}	0.58^{b}	3.72^{c}	0.13^{abc}
15	80	-	-	4.34^{ab}	0.90^{a}	0.67^{ab}	4.78^{a}	0.15^{a}
		-	+	3.91^{bc}	0.70^{b}	0.61^{ab}	3.78^{c}	0.13^{abc}
		+	-	3.99^{bc}	0.74^{b}	0.67^{ab}	3.91^{bc}	0.13^{abc}
		+	+	3.70^{c}	0.73^{b}	0.63^{ab}	3.75^{c}	0.12^{bc}
30	50	-	-	4.58^{ab}	0.82^{ab}	0.69^{ab}	4.56^{a}	0.15^{ab}
		-	+	4.37^{ab}	0.70^{b}	0.65^{ab}	4.36^{ab}	0.10^{c}
		+	-	4.17^{bc}	0.82^{ab}	0.58^{b}	3.61^{c}	0.13^{abc}
		+	+	3.80^{bc}	0.76^{b}	0.63^{ab}	3.84^{bc}	0.12^{abc}
30	80	-	-	4.84^{a}	0.88^{ab}	0.65^{ab}	4.69^{a}	0.12^{abc}
		-	+	4.41^{ab}	0.75^{b}	0.72^{a}	4.51^{a}	0.13^{abc}
		+	-	4.20^{b}	0.85^{ab}	0.72^{a}	4.33^{ab}	0.14^{abc}
		+	+	4.05^{bc}	0.77^{ab}	0.71^{a}	4.44^{ab}	0.11^{bc}
			SEM	0.19	0.05	0.04	0.20	0.01
Source of variation								
Temperature				***	NS	NS	**	NS
Relative humidity				**	NS	*	**	NS
Inhibitor				***	NS	NS	***	NS
Detoxifier				***	***	NS	**	*
Temp x RH				NS	NS	NS	NS	NS
Temp x Inhibitor				NS	*	NS	NS	NS
RH x Inhibitor				NS	NS	**	NS	NS
Temp x RH x Inhibitor				NS	NS	NS	NS	NS

[a,b,c,d] Mean values in the same column not sharing a superscript are significantly different

p<0.05, ** p<0.01 & *** p<0.001, NS not significant.

SEM is the standard error of the means.

Birds that consumed feeds stored at low temperature had significantly ($p<0.001$) lower liver weights than those, which were on diets stored at high temperature. Similarly, low storage RH significantly ($p<0.01$) reduced liver weight of birds. Inclusion of the inhibitor in diets significantly ($p<0.001$) depressed liver weight. A similar reduction ($p<0.001$) in the weight of liver was observed when the detoxifier was included in the diets. Interactions between the factors had no significant effect on liver weight.

Storage temperature, RH and inclusion of inhibitor did not significantly influence heart weight (Table 4.4). Furthermore, inclusion of the inhibitor in the diet did not significantly affect heart weight, although heart weight was slightly reduced. The presence of the detoxifier in the diet reduced ($p<0.001$) heart weight of birds by 11%. Apart from temperature x inhibitor interaction ($p<0.05$), no other level of interaction studied affected heart weight. Storage temperature did not significantly affect proventriculus weight but it was found that storage RH was found to significantly influence ($p<0.001$) proventriculus weight by 4%. Inclusion of the inhibitor or detoxifier in diets had no effect on proventriculus weight of birds. RH x inhibitor interaction significantly ($p<0.01$) affected proventriculus weight (Table 4.4).

Table 4.4 reveals that diets stored at low temperature or RH, significantly ($p<0.01$) decreased the gizzard weights in birds. Inclusion of the inhibitor in the diets also significantly reduced ($p<0.001$) gizzard weight by 4%. Similarly, gizzard weight was reduced ($p<0.01$) by about 6% due to the presence of the detoxifier in the diets stored at low temperature. None of the levels of interactions tested significantly influenced gizzard weight. Storage temperature and RH had no significant effect on spleen weight. Furthermore, inclusion of the inhibitor in the diet did not significantly affect spleen weight. Conversely, inclusion of a detoxifier in the diet significantly ($p<0.05$) reduced

spleen weight. Similar to the gizzard, interactions between the factors had no effect on spleen weight.

4.1.7 Serum biochemistry

Results of the effects of temperature, relative humidity, inhibitor and detoxifier on the biochemistry of serum of broiler chicks after 21 days are presented in Table 4.5. Storage temperature and RH had no significant effect on total phosphorus. The presence of the inhibitor also did not significantly increase serum PO_4. However, inclusion of the detoxifier increased ($p<0.001$) the concentration of serum PO_4 in birds by 3.5%. In the absence of the detoxifier, temperature x RH x inhibitor interaction significantly affected ($p<0.05$) PO_4 level (Table 4.5). No other interactions studied had any significant effect on the level of PO_4.

Storage temperature and RH did not significantly affect serum protein content. Inclusion of the inhibitor in diets, however, resulted in an increase of 6% ($p<0.05$) in serum protein. Unlike the inhibitor, the toxin-binder (detoxifier) had no effect on serum protein content. Temperature x RH x inhibitor was the only interaction that had a ($p<0.001$) significant effect on serum protein.

Albumin content was not affected by any of the factors studied. Furthermore, no level of interaction had a significant effect on albumin content except for the fact that temperature x RH x inhibitor had a significant effect ($p<0.05$) on the concentration of serum albumin (Table 4.5). The concentration of globulin in sera of birds followed a similar pattern to total phosphorus, serum protein and albumin, with no significant effects of storage temperature or RH. Inclusion of the inhibitor in diets significantly

($p<0.01$) improved globulin content. The detoxifier had no significant effect on globulin level in the sera of birds. Temperature x RH and temperature x RH x inhibitor had a significant effect ($p<0.05$) on globulin level (Table 4.5)

Table 4.5 Effect of varying storage temperature, relative humidity, inhibitor /antioxidant and detoxifier on phosphate, serum protein, albumin and globulin.

Temp	RH	Inhib.	Detox.	Phos.	Ser.Prot	Alb.	Glob
15	50	-	-	2.88^{d}	3.13^{bc}	1.50^{ab}	1.64^{b}
		-	+	3.56^{bcd}	3.21^{bc}	1.73^{a}	1.47^{b}
		+	-	3.65^{abcd}	3.21^{bc}	1.45^{ab}	1.76^{ab}
		+	+	3.58^{bcd}	2.90^{bc}	1.35^{ab}	1.55^{b}
15	80	-	-	3.29^{cd}	2.86^{c}	1.27^{b}	1.59^{b}
		-	+	4.21^{ab}	3.12^{bc}	1.48^{ab}	1.64^{b}
		+	-	3.48^{bcd}	3.27^{bc}	1.47^{ab}	1.80^{ab}
		+	+	3.55^{bcd}	3.51^{ab}	1.52^{ab}	1.99^{ab}
30	50	-	-	3.00^{cd}	3.02^{bc}	1.58^{ab}	1.45^{b}
		-	+	4.42^{a}	2.88^{bc}	1.32^{b}	1.56^{b}
		+	-	3.12^{cd}	3.14^{bc}	1.42^{ab}	1.72^{b}
		+	+	2.93^{cd}	3.88^{a}	1.66^{ab}	2.22^{a}
30	80	-	-	2.99^{cd}	3.05^{bc}	1.48^{ab}	1.57^{b}
		-	+	3.76^{abc}	3.38^{b}	1.68^{ab}	1.70^{b}
		+	-	3.12^{cd}	3.07^{bc}	1.41^{ab}	1.66^{b}
		+	+	3.44^{bcd}	3.05^{bc}	1.53^{ab}	1.52^{b}
		SEM		0.28	0.18	0.15	0.17
Source of variation							
Temperature				NS	NS	NS	NS
Relative humidity				NS	NS	NS	NS
Inhibitor				NS	*	NS	**
Detoxifier				***	NS	NS	NS
Temp x RH				NS	NS	NS	*
Temp x Inhibitor				NS	NS	NS	NS
RH x Inhibitor				NS	NS	NS	NS
Temp x RH x Inhibitor				*	***	***	*

[a,b,c,d] Mean values in the same column not sharing a superscript are significantly different $p<0.05$, ** $p<0.01$ & *** $p<0.001$ NS not significant.
SEM is the standard error of the means.

4.1.8 Tibia ash

Data obtained from tibia ash analysis were not statistically analysed and therefore, results are stated in absolute values as shown in Table 4.6. Results demonstrate that tibia ash of birds was improved when diets were stored at low RH in high temperature environment. At high temperature, inclusion of an inhibitor as well as a detoxifier increased tibia ash.

Table 4.6 Effect of varying storage temperature, relative humidity, inhibitor/antioxidant and detoxifier on tibia ash (absolute values).

Temp (°C)	RH (%)	Inhib.	Detox.	Tibia ash (%)
15	50	-	-	52.6
		-	+	52.6
		+	-	51.7
		+	+	51.0
15	80	-	-	51.0
		-	+	51.8
		+	-	51.4
		+	+	52.4
30	50	-	-	50.5
		-	+	51.1
		+	-	50.5
		+	+	51.1
30	80	-	-	50.0
		-	+	51.3
		+	-	51.7
		+	+	52.6

Samples were pooled and analysed in duplicate as described in Section 3.8.4
Data presented are laboratory results. No statistical analyses were performed.

4.2 Discussion

4.2.1 Nutrient composition

Storage of diets at high temperature (30°C) resulted in an increase in the DM content, which further resulted in an increase in CP as well as fat contents of the diets after one

month of storage. This is due to initial loss in moisture content. Storage at low temperature in a high RH environment resulted in a slight decrease in DM. Diets stored under such a condition may have absorbed moisture from the surrounding atmosphere. After two months of storage, a drop in DM content was observed in all diets apart from those stored at high temperature and low RH. It was likely that diets at other conditions imbibed moisture from the environment but the reduction may also be due to mold growth and development (Njobeh, 1997).

The increase in the concentration of Fe and Zn after one month of storage followed a similar trend to that of fat and CP, which may be correlated to the link between DM and other nutrients as earlier stated. However, the concentration of Cu dropped remarkably throughout storage. Transition metals are pro-oxidants, facilitating lipid oxidation as catalysts (Decker and Zhinin, 1998; Ruiz *et al.*, 2000). If further fungal growth occurred during storage, some of the nutrients could also be lost in form of spores. However, it is also possible that Cu may have leached out of the diets with prolonged storage at high temperature (Lang, 1970). Decker and Zhinin (1998) suggested that loss of transition metals in diets might be due to the presence of antioxidants. In regards to the reduction in the concentration of Cu, no conclusion can be established, from the results of this study.

4.2.2 Feed microbiology

Microbiological analysis indicated that no toxins were detected and that *Aspergillus spp, Penicillium spp* as well as *Fusarium spp* were the fungi identified in the diet prior to storage. Although these fungi are the most predominant in South Africa (Devegowda *et al.*, 1998) and are found in maize-containing feeds (Dutton and Kinsey, 1996), it is

likely that the toxins tested were below detection limits or the ingredients used in the formulation of the diets might have been fresh or well preserved to limit microbial growth and production of mycotoxins.

4.2.3 Rancidity

Fat deterioration (hydrolysis and oxidation) was much more evident at high temperature (30°C) than at low temperature (15°C). This is proven by higher concentrations of FFA and Pv in diets stored at high temperature than at low temperature. This confirms a report by van den Berghe (1990) who stated that fats are unstable at high temperature. High RH also increased hydrolysis as well as oxidation of fat in the diets. High RH is known to provide a favourable condition for hydrolysis and to a lesser extent, oxidation of fat because moisture is being absorbed from the atmosphere. In addition, there is high potential for O_2 to diffuse into the diets with high water concentration at a faster rate, thus effecting rapid lipid peroxidation.

Oxidation and hydrolytic stability of fat in diets are influenced by antioxidants. Vitamin E was shown to reduce the rate of fat oxidation as observed in earlier studies (Bautista *et al.*, 1992). The mechanism involved in the prevention of oxidation might have been through deactivation of free radicals or possibly through the formation of complex compounds with transition metals and render the metals less active as pro-oxidants (Decker and Zhinin, 1998). It was observed that fat hydrolysis was not significantly suppressed as confirmed in most reports, which state that antioxidants do not prevent or inhibit the hydrolysis of fat (Coppen, 1989). Antioxidants are known to prevent the action of free radicals produced during the first phase of oxidation, which are not evident during hydrolysis. Rapid changes associated with fat deterioration are a

common phenomenon. High temperature was observed to reduce the capabilities of vitamin E to prevent oxidation. This may be explained by the fact that vitamin E is itself unstable and is oxidized (Paster *et al.*, 1985; Lin *et al.*, 1989) or leached away (Lang, 1970) when subjected to heat over prolonged periods causing a significant increase in the rate of peroxidation in diets.

4.2.4 Production parameters

The effects of temperature, RH, inhibitor and toxin-binder on feed intake were inconsistent and less conclusive. It was expected that diets stored at high temperature would undergo deteriorative changes leading to off-flavour taste and odour, leading to a low feed intake as observed in previous studies (Lin *et al.*, 1989; Sanders, 1989; Bautista, 1992) or may lead to complete feed refusal (Galliard, 1989). However, in this study, this was not the case, as feed intake was not influenced by any of the factors. Diet supplementation with the antioxidant to suppress oxidation did not have any influence on feed intake. It is probable that the concentration of FFA and Pv in the diets did not attain critical levels to induce loss of appetite or feed refusal. In addition to antioxidant, it is probable that the mold inhibitor that was included in the diets might have suppressed the growth of fungi and possible production of mycotoxins. At high temperature, it was found that the antioxidant significantly improved FI, which was not noticeable at low temperature. This might be an indication that high temperature increases the rate of oxidation, which was suppressed in the presence of an antioxidant. Intake of these diets was markedly improved with detoxifier supplementation.

Unlike feed intake, body weight was significantly decreased by storage of diets at high temperature. Low body weight of birds fed diets stored at high temperature in high RH

environment confirms the high FFA and Pv levels in feeds stored under such a condition. Birds that consumed such diets might have had diarrhoea as possible biological symptom of consumption of rancid feeds (Sanders, 1985) leading to poor growth (Shermer and Calabotta, 1985). Results indicate that supplementation of vitamin E or Moldzab did not significantly improve body weight of birds. However, it is unlikely that the deleterious effects of rancid feeds or toxins might have been alleviated when diets were supplemented with the inhibitor. These suggestions are in agreement with those of Cabel and Waldroup (1988) who stated that vitamin E was effective in controlling encephalomalacia in chicks.

Supplementation of toxin-binder in diets significantly improved body weight regardless of storage temperature and RH. A variety of sorbent materials have been widely used in preventing the occurrence of mycotoxicosis in birds. Previous reports indicate that the detoxifier used in this study (Mycosorb) has been shown to be the most effective (Valarezo *et al.*, 1997; Devegowda *et al.*, 2000). The efficacy of Mycosorb to suppress the biological effects of rancid feeds is not known. However, it is probable that promotion of body weight in birds can be attributed to the capabilities of Mycosorb to bind toxins and prevent their absorption in the gastrointestinal tract, thereby reducing their toxic effects (Valarezo *et al.*, 1997; Raju and Devegowda, 2000). The most probable conclusion is that Mycosorb alleviated the biological effects of toxins, which might have been produced alongside FFA and peroxides in feeds.

High storage temperature and RH depressed FCE. High RH (80%) as observed, significantly increased the concentrations of FFA as well as Pv. The diets stored at high temperature and high RH were the least efficient to be converted into animal protein.

However, this condition was reversed when diets were supplemented with vitamin and mold inhibitor. Further supplementation with the detoxifier significantly increased FCE (more evident at high temperature). The beneficial effect of detoxifier in deactivating toxins (Smith, 1999) has been well established. Similar effects have been reported on body weight as well as FCE. Increased body weight and FCE by supplemental detoxifier may have been mediated by efficient absorption and utilisation of nutrients (van den Berghe, 1990). It is also a possibility that mold inhibitor (partly composed of vitamin C) may have stimulated the immune system in preventing mycotoxicosis in chicks leading to increased body weight and FCE.

4.2.5 Visceral organ weight

This study demonstrated that high storage temperature and RH significantly increased, liver and gizzard weights of birds indicating that there was probably a much higher production of toxins in diets stored under such conditions. Addition of the mold inhibitor in diets was effective in reducing organ weights (liver and gizzard). It might have been that toxin production in the diets was suppressed when supplemental mold inhibitor was introduced. Results also demonstrated the ability of the detoxifier in decreasing organ weight except for the weight of the proventriculus. The toxins might have been detoxified and eliminated while in the GIT (Devegowda *et al.*, 2000).

4.2.6 Serum biochemistry

Significant differences were obtained in the concentration of serum phosphorus when diets were supplemented with the detoxifier. Birds that were offered diets supplemented with a detoxifier had higher phosphorus content than those without detoxifier. Similar results have been reported in ducklings as well as broilers on the possible effects of the

detoxifier against aflatoxicosis (Khajarern and Khajarern, 1999). It may be possible that the detoxifier might have improved phosphorus absorption.

Concentration of serum protein was significantly increased in birds fed diets supplemented with the mold inhibitor. Although significant differences were not observed, serum protein concentration was found to increase when diets were supplemented with the detoxifier. Similar results have been reported by Raju and Devegowda (2000) and Abdel-wahhab *et al.* (2002). It is likely that supplemental detoxifier prevents the impairment of protein synthesis by toxins (Bailey *et al.* 1998; Kubena *et al.*, 1998) thereby increasing its concentration in serum. Serum albumin was significantly improved when diets were supplemented with the inhibitor, which probably prevented the development of fungi and possible production of mycotoxins.

4.2.7 Tibia ash

There are very few reports on the effects of mycotoxins on bone ash. In the present study, bone ash appeared to be negatively affected by the inhibitor that should have protected vitamin D_3 in the diets, unlike the detoxifier, which enhanced tibia ash. Bone ash content is reported to be influenced by ochratoxin (Huff *et al.*, 1977, cited by Raju and Devegowda, 2000) by interfering with vitamin D_3 metabolism, which is actively involved in bone mineralisation. However, ochratoxin is uncommon in South Africa and therefore the absence of an incidence of rubbery bone in this study is obvious.

4.3 Conclusion

This trial clearly demonstrated an increase in the rates of peroxidation and hydrolysis of fats in feeds stored at high temperature and high relative humidity, in which the former

was markedly reduced in the presence of an antioxidant. The study also revealed the efficacy of Mycosorb in preventing mycotoxicosis or rancid feed effects in broiler chicks, which further enhanced their production performance.

CHAPTER FIVE

5.0 Effects of storage conditions and supplements on nutrient composition and nutritive value of high-lipid maize-based diets for broiler chickens (Experiment Two).

5.1 Results

5.1.1 Chemical composition of the diets

The chemical composition of the test diets is presented in Table 5.1 and Fig. 5.1. There was a slight increase in the DM content of the diets except in diets stored at low temperature and high RH in which there was a drop in the DM content after one month of storage. The increase in DM was most pronounced in diets stored at high temperature

Table 5.1 Nutrient compositions (g/kg) of the fresh diets

Nutrient	Composition
Dry matter	883.0
Crude protein	204.0
Fat	49.0
Crude fibre	28.0
Calcium	15.0
Phosphorus	8.0
Methionine	2.0
Lysine	9.0
AME_n (MJ/kg)	12.5
TME_n (MJ/kg)	12.9
Iron (mg/kg)	280.0
Zinc (mg/kg)	180.0
Copper (mg/kg)	30.0

and low RH environment. The diets varied more widely in the DM content after two months of storage. The increase in CP level was most noticeable in diets with the

inhibitor after one month of storage, while at two months, there was a wider variation in CP content of the diets. Little or no change in fat content was observed in diets at low temperature regardless of storage RH while at high temperature, fat content of the diets was substantially increased after one month. After two months of storage, there was a decrease in fat level except in diets stored at low temperature and high RH (Fig. 5.1).

Micro-mineral (Fe, Zn and Cu) contents of the diets decreased after one month of storage, with Cu experiencing a sharp decrease (Fig. 5.1). There was some fluctuation in the concentrations of Fe and Zn content with time. The rate of decrease in the concentration of Cu was higher over one month than over two months of storage. In addition, the decrease in the concentration of Cu was most noticeable in diets devoid of inhibitor throughout storage.

5.1.2 Feed microbiology

Microbiological analyses of feed samples prior to storage showed some fungi. The species that were detected include *Aspergillus flavus*, *A parasiticus, Penicillium spp* and *Fusarium moniliformes*. Mycotoxicological findings revealed no toxins.

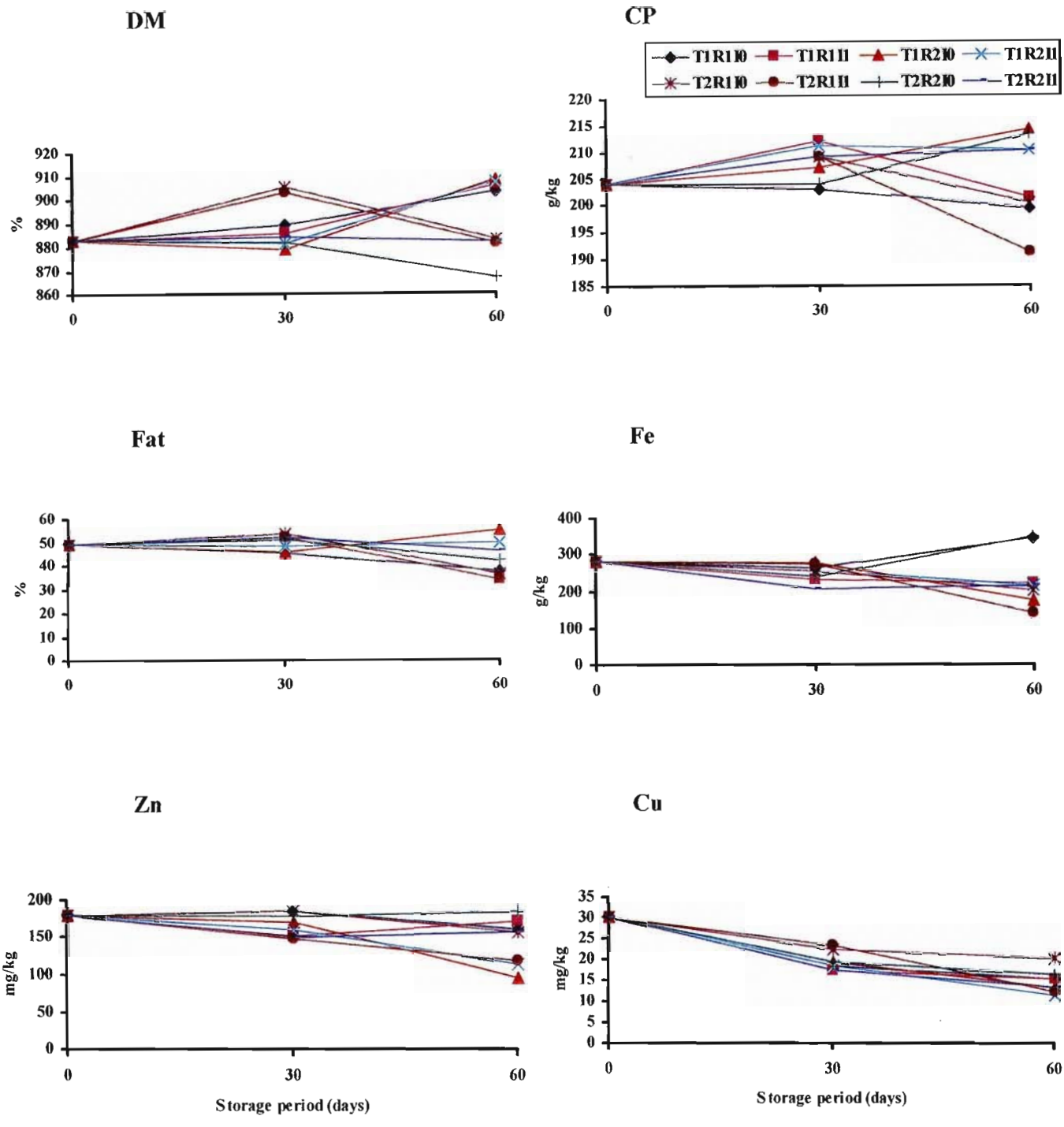

Figure. 5.1. Changes in the nutrient composition of the diets with time. T1, 15°C; T2, 30°C; R1, RH 50%; R2, RH 80%; I0, without inhibitor; I1, with inhibitor. Samples were analysed in duplicates and mean values derived. No statistical analysis was performed.

5.1.3 Rancidity

The free fatty acid contents of the diets rapidly increased ($p<0.001$, $R^2= 0.84$) from an initial value of 3.8 to an average value of about 14% over the experimental period of 60 days (Table 5.2 and Appendix VI). Storage of diets at high temperature significantly ($p<0.001$) increased FFA content to about 15%. The FFA levels in diets stored under such conditions were consistently higher throughout the storage period than those of the other diets.

High storage temperature enhanced the rate of formation of FFA. In addition, there was a significant increase ($p<0.001$) in FFA concentration in the diets as a result of high RH and low temperature. Inclusion of the inhibitor in the diets at low temperature reduced ($p<0.05$) the rate of hydrolysis of fats after one month of storage (Table 5.2) while after two months of storage, no variation was observed in the concentration of FFA in diets with or without inhibitor. The greatest concentrations of FFA were observed in diets stored at high temperature with or without inhibitor. Various levels of interactions among the four factors affected ($p<0.05$) FFA in the diets.

The peroxide value of the diets followed a trend similar to that of FFA except for the fact that the rate of peroxidation in the feeds was significantly decreased ($p<0.001$) with an inclusion of the inhibitor (Table 5.2). The presence of the inhibitor reduced the concentration of peroxides in the diet by about 40% by the end of the storage period. The Pv of the diets rapidly increased ($p<0.001$, $R^2 = 0.89$) from an initial value of 0.6 to an average of about 6.5 mEq/kg fat throughout the experimental period of 60 days (Table 5.2 and Appendix VI). Storage at high temperature significantly ($p<0.001$) increased Pv with increasing length of storage period. The highest Pv were obtained in diets stored under such conditions throughout the storage period

Table 5.2. Effects of varying feed storage conditions and antioxidant inclusion on the concentration of free fatty acids (%) and peroxides (mEq O_2/kg fat)

Temp	RH	Inhibitor	Period	FFA	PV
15	50	-	1	3.8^{j}	0.6^{k}
			2	6.7^{h}	3.2^{h}
			3	10.7^{f}	6.1^{d}
		+	1	3.6^{j}	0.6^{k}
			2	5.6^{i}	2.1^{j}
			3	10.7^{f}	4.7^{f}
15	80	-	1	3.8^{j}	0.6^{k}
			2	8.3^{g}	3.6^{g}
			3	12.9^{cd}	6.1^{d}
		+	1	3.6^{j}	0.6^{k}
			2	8.3^{g}	2.6^{i}
			3	12.8^{d}	5.2^{e}
30	50	-	1	3.8^{j}	0.6^{k}
			2	10.6^{f}	4.5^{f}
			3	13.6^{c}	7.9^{b}
		+	1	3.6^{j}	0.6^{k}
			2	10.3^{f}	2.7^{i}
			3	14.3^{b}	6.5^{c}
30	80	-	1	3.8^{j}	0.6^{k}
			2	11.5^{e}	4.8^{f}
			3	16.9^{a}	8.5^{a}
		+	1	3.6^{j}	0.6^{k}
			2	10.8^{ef}	3.2^{h}
			3	17.5^{a}	6.6^{i}
	SEM			0.20	0.05
Source of variation					
Temp				***	***
RH				***	***
Inhibitor				*	***
Period				***	***
Temp*RH				NS	NS
Temp*Inhibitor				*	***
Temp*Period				***	***
RH*Inhibitor				NS	NS
RH*Period				***	***
Inhib*Period				***	***
Temp*RH*Inhib				*	***
Temp*RH*Period				***	NS
Temp*Inhibitor*Period				*	***
RH*Inhibitor*Period				NS	NS
Temp*RH*Inhibitor*Period				*	***

^{a,b,c,d,e,f,g,h,I,j} Mean values in the same column not sharing a superscript are significantly different p<0.05, ** p<0.01 & *** p<0.001 NS, not significant.
SEM is the standard error of difference between the mean values.

Levels of interactions were observed to have significant effects ($p<0.001$) on the concentration of peroxides in the diets except temperature x RH, RH x inhibitor, temperature x RH x time and RH x inhibitor x time interactions, which did not influence the Pv of the diets.

5.1.4 Production parameters

The effects of temperature, relative humidity, inhibitor and mycotoxin-binder on the performance of broiler chicks were studied and results are presented in Table 5.3. Feed intake was not statistically affected by any of the factors during the 21-day trial period. Feeds stored at low RH significantly ($p<0.01$) increased body weight by 3%. Similarly, birds that were fed on diets stored at low temperature had significantly ($p<0.01$) higher body weights than those that were on diets stored at high temperature. Inclusion of a detoxifier significantly ($p<0.001$) improved the chicks' body weight at 21 days (Table 5.3).

Unlike body weight, feed conversion efficiency was not significantly affected by feed storage temperature although there was some tendency for FCE of birds that consumed diets stored at low temperature to be higher than that of birds on feeds stored at high temperature. Storage of feeds at low RH significantly ($p<0.01$) increased FCE. No statistical differences in FCE were observed when the inhibitor was included in diets. In addition, birds that were fed diets with the detoxifier did not show any improvement in FCE. There were no significant interactions among factors on FCE except that of temperature x RH ($p<0.05$).

Table 5.3. Effect of varying storage temperature, relative humidity, inhibitor/antioxidant and detoxifier on feed intake (g/bird), final body weight (g) and feed conversion efficiency (g weight gain/kg feed).

Temp (°C)	RH (%)	Inhib.	Detox.	Feed intake	Body weight	FCE
15	50	-	-	947	630^{abc}	607^{ab}
		-	+	927	660^{a}	652^{a}
		+	-	944	647^{abc}	624^{ab}
		+	+	963	655^{a}	625^{ab}
15	80	-	-	917	632^{abc}	629^{ab}
		-	+	970	645^{abc}	608^{ab}
		+	-	933	623^{abc}	608^{ab}
		+	+	946	635^{abc}	613^{ab}
30	50	-	-	919	643^{abc}	641^{ab}
		-	+	911	652^{ab}	654^{a}
		+	-	895	603^{bc}	613^{ab}
		+	+	899	641^{abc}	651^{a}
30	80	-	-	943	600^{c}	578^{b}
		-	+	957	617^{abc}	587^{b}
		+	-	937	611^{abc}	594^{ab}
		+	+	935	620^{abc}	604^{ab}
			SEM	31.52	17.94	24.19
Source of variation						
Temperature				NS	**	NS
Relative humidity				NS	**	**
Inhibitor				NS	NS	NS
Detoxifier				NS	**	NS
Temp x RH				NS	NS	*
Temp x Inhibitor				NS	NS	NS
RH x Inhibitor				NS	NS	NS
Temp x RH x Inhibitor				NS	NS	NS

[a,b,c] Mean values in the same column not sharing a superscript are significantly different p<0.05, ** p<0.01 & *** p<0.001 NS not significant.
SEM is the standard error of the means.

5.1.5 Mortality

There was no pattern in the mortality recorded, on account of treatment effects. Overall mortality in this was study was about 6.5%.

5.1.6 Visceral organ weight

The effects of temperature, relative humidity, inhibitor and mycotoxin-binder on the relative visceral organ weight of broiler chicks were studied and results are presented in Table 5.4.

Birds that consumed diets stored at low temperature had significantly ($p<0.05$) lower liver weights than those on diets stored at high temperature. When the inhibitor or detoxifier was included in the diets, liver weight was significantly reduced ($p<0.001$) by about 9%. Storage RH did not significantly affect liver of birds. Levels of interactions that were studied showed that only temperature x RH x inhibitor influenced ($p<0.05$) the liver weights of birds. Neither storage temperature nor RH had a significant effect on the heart weight of birds. Unlike the inhibitor, the presence of the detoxifier in diets irrespective of storage conditions, significantly ($p<0.01$) reduced heart weight (11%). This was much more noticeable at low temperature. Various levels of interactions tested did not show any significant effect on heart weight as shown in Table 5.4.

Proventriculus weight was not significantly affected by any of the factors. In addition, the presence of the detoxifier in diets did not depress proventriculus weight of birds. Similar to the heart and proventriculus, gizzard weight was not statistically affected by storage temperature or RH (Table 5.4). The inhibitor in the diets significantly ($p<0.05$) depressed gizzard weight. Also, gizzard weight was significantly reduced ($p<0.001$) by the detoxifier. No levels of interactions were found to significantly influence gizzard weight. None of the factors studied had a significant effect on spleen weight. Interaction

of RH x inhibitor significantly ($p<0.05$) affected spleen weight. No other levels of interactions affected spleen weight.

Table 5.4. Effect of varying storage temp (°C), RH (%), inhibitor/antioxidant and detoxifier on the relative weight of visceral organs.

Temp	RH	Inhib.	Detox.	Liver	Heart	Prov.	Giz.	Spleen
15	50	-	-	4.31^{ab}	0.97^{a}	0.72^{abc}	4.57^{a}	0.14^{abc}
		-	+	3.56^{cd}	0.69^{c}	0.63^{bc}	3.71^{de}	0.13^{bc}
		+	-	4.10^{bcd}	0.81^{bc}	0.75^{ab}	4.52^{ab}	0.11^{c}
		+	+	3.55^{cd}	0.69^{c}	0.63^{bc}	3.85^{cde}	0.13^{bc}
15	80	-	-	4.76^{a}	0.77^{bc}	0.69^{abc}	4.35^{ab}	0.15^{abc}
		-	+	3.98^{bcd}	0.80^{bc}	0.65^{abc}	4.08^{cd}	0.13^{bc}
		+	-	3.52^{cd}	0.85^{abc}	0.71^{abc}	4.18^{bc}	0.17^{a}
		+	+	3.40^{d}	0.68^{c}	0.67^{abc}	3.85^{cde}	0.12^{bc}
30	50	-	-	4.20^{abc}	0.87^{ab}	0.67^{abc}	4.34^{ab}	0.12^{bc}
		-	+	4.28^{ab}	0.74^{bc}	0.77^{a}	4.12^{bc}	0.17^{a}
		+	-	4.10^{bc}	0.82^{b}	0.69^{abc}	4.12^{bcd}	0.13^{bc}
		+	+	3.53^{cd}	0.74^{bc}	0.59^{c}	3.60^{e}	0.12^{bc}
30	80	-	-	4.54^{ab}	0.73^{c}	0.69^{abc}	4.25^{ab}	0.14^{abc}
		-	+	4.53^{ab}	0.80^{bc}	0.76^{ab}	4.41^{ab}	0.11^{bc}
		+	-	4.20^{abc}	0.78^{bc}	0.76^{ab}	4.12^{bc}	0.15^{ab}
		+	+	3.68^{cd}	0.80^{bc}	0.73^{ab}	4.22^{abc}	0.12^{bc}
			SEM	0.24	0.04	0.05	0.05	0.01
Source of variation								
Temperature				*	NS	NS	NS	NS
Relative humidity				NS	NS	NS	NS	NS
Inhibitor				***	NS	NS	*	NS
Detoxifier				***	**	NS	***	NS
Temp x RH				NS	NS	NS	NS	NS
Temp x Inhibitor				NS	NS	NS	NS	NS
RH x Inhibitor				NS	NS	NS	NS	*
Temp x RH x Inhibitor				*	NS	NS	NS	NS

[a,b,c,d] Mean values in the same column not sharing a superscript are significantly different p<0.05, ** p<0.01 & *** p<0.001 NS not significant.
SEM is the standard error of the means.

5.1.7 Serum biochemistry

Results of the effects of temperature, relative humidity, inhibitor and detoxifier on the biochemistry of serum of the broiler chicks are presented in Tables 5.5. Unlike storage

RH, temperature significantly ($p<0.05$) increased serum phosphate. Inclusion of the inhibitor in diets did not significantly affect PO_4 content. In contrast, the detoxifier was effective in increasing ($p<0.05$) PO_4 level in serum by 3.5%. All levels of interactions tested showed some significant effects. It was observed that PO_4 was significantly affected by temperature x inhibitor and RH x inhibitor (at $p<0.01$) and by temperature x RH and temperature x RH x inhibitor (at $p<0.05$) (Table 5.5).

Storage temperature had no significant effect on serum protein concentration in birds. Conversely, Low storage RH significantly ($p<0.05$) increased serum protein. Inclusion of the inhibitor and detoxifier in diets did not significantly influence the concentration of serum protein. Temperature x RH ($p<0.05$) and temperature x inhibitor ($p<0.01$) had significant effects on serum protein (Table 5.5). No other levels of interactions affected significantly the serum protein content.

Storage temperature and RH did not influence serum albumin level. Inclusion of the inhibitor as well as the detoxifier significantly ($p<0.05$) increased albumin concentration in serum. RH x inhibitor interaction also significantly ($p<0.05$) influenced serum albumin. Unlike storage temperature, low RH significantly ($p<0.05$) increased globulin content in sera of birds. Inclusion of either an inhibitor or detoxifier significantly increased globulin level. Of the interactions tested, only temperature x inhibitor influenced ($p<0.01$) globulin level.

Table 5.5 Effect of varying storage temperature (°C), relative humidity (%), inhibitor/antioxidant and detoxifier on serum phosphate, protein, albumin and globulin.

Temp	RH	Inhib.	Detox.	Phos.	Ser. Prot.	Alb.	Glob.
15	50	-	-	3.35^{ab}	2.90^{c}	1.34^{b}	1.56^{bc}
		-	+	3.60^{ab}	3.36^{ab}	1.40^{b}	1.96^{a}
		+	-	3.16^{bc}	3.52^{a}	1.57^{ab}	1.95^{ab}
		+	+	3.20^{abc}	3.53^{a}	1.61^{ab}	1.92^{ab}
15	80	-	-	3.66^{ab}	3.23^{ab}	1.56^{ab}	1.68^{abc}
		-	+	3.54^{ab}	2.66^{c}	1.31^{b}	1.35^{d}
		+	-	3.20^{ab}	3.04^{bc}	1.51^{ab}	1.52^{cd}
		+	+	3.74^{a}	3.20^{ab}	1.41^{b}	1.79^{abc}
30	50	-	-	3.20^{ab}	2.84^{c}	1.24^{b}	1.61^{abc}
		-	+	3.57^{ab}	3.52^{a}	1.57^{ab}	1.95^{a}
		+	-	3.24^{ab}	3.05^{bc}	1.48^{ab}	1.56^{abc}
		+	+	3.24^{ab}	3.10^{b}	1.68^{ab}	1.42^{cd}
30	80	-	-	2.81^{c}	3.22^{ab}	1.49^{ab}	1.73^{abc}
		-	+	2.97^{bc}	3.15^{ab}	1.64^{ab}	1.51^{cd}
		+	-	3.50^{ab}	3.03^{bc}	1.34^{b}	1.69^{abc}
		+	+	3.62^{ab}	3.08^{bc}	1.70^{a}	1.38^{cd}
			SEM	0.61	0.44	0.10	0.14
Source of variation							
Temperature				*	NS	NS	NS
Relative humidity				NS	*	NS	*
Inhibitor				NS	NS	*	NS
Detoxifier				*	NS	*	NS
Temp x RH				*	*	NS	NS
Temp x Inhibitor				**	**	NS	**
RH x Inhibitor				**	NS	*	NS
Temp x RH x Inhibitor				*	NS	NS	NS

[a,b,c,d] Mean values in the same column not sharing a superscript are significantly different p<0.05, ** p<0.01 & *** p<0.001 NS not significant.
SEM is the standard error of the means.

5.1.8 Tibia ash

Data obtained from tibia ash analysis were not statistically analysed and therefore, results are stated in absolute values as shown in Tables 5.6. Results indicate that storage

temperature did not influence tibia ash content. In addition, tibia ash was not affected by storage RH although it was observed that at high temperature (30°C), low RH increased tibia ash content of birds by about 3%. Inclusion of the inhibitor at high temperature was found to increase tibia ash of birds. Irrespective of storage temperature and RH, birds that were fed on diets with detoxifier had higher tibia ash content than those without detoxifier.

Table 5.6 Effect of varying storage temperature, relative humidity, inhibitor/antioxidant and detoxifier on tibia ash (absolute values).

Temp (°C)	RH (%)	Inhib.	Detox.	Tibia ash (%)
15	50	-	-	50.7
		-	+	52.0
		+	-	51.0
		+	+	51.4
15	80	-	-	51.1
		-	+	52.0
		+	-	50.5
		+	+	53.1
30	50	-	-	50.8
		-	+	52.4
		+	-	50.9
		+	+	55.3
30	80	-	-	49.8
		-	+	50.5
		+	-	50.1
		+	+	52.9

Samples were pooled and analysed in duplicate as described in Section 3.8.4. No statistical analysis was done.

5.2 Discussion

5.2.1 Nutrient composition

There was an increase in the DM content as a result of storage over one month, which was accompanied by an increase in CP as well as fat content. There was a further

increase in the level of CP in the diets stored at high RH but a drop in CP content was experienced in diets stored at low RH while fat content in most diets decreased after two months of storage. This drop in fat content may be as a result of rancidity and (or) volatilisation of fatty acids. A direct relationship exists between DM and other nutrients, especially protein and lipids (Iji *et al.*, 2003). As the moisture content of the diet decreases, the concentration of the other nutrients also increases provided there is no volatilisation of nutrients such as lipids or amino acids. The changes in the DM content after two months of storage were difficult to explain. Some of the diets stored at low temperature increased in DM while certain diets at high temperature had low DM. High temperature may cause a loss of fatty acids or other nutrients as suggested by Iji *et al.* (2003). This rate of volatilisation may have been accentuated by a high dietary lipid content.

The concentration of micro-minerals (Fe and Zn) decreased after one month of storage while in the second month, further reductions were observed in most of the treatments. The concentration of Cu decreased throughout storage. This decrease in the concentration of transition metals may be attributed to leaching (Lang, 1977), or the presence of antioxidants could have contributed to a reduction in the concentrations of these minerals in the diets as suggested by Decker and Zhinin (1998). Similar changes were observed in the first experiment.

5.2.2 Rancidity

In agreement with other studies (Bautista *et al.*, 1992; Ramezanzadeh *et al.*, 1999), fat deterioration occurred throughout the storage period as observed in experiment one. Hydrolysis and oxidation of fat were higher in diets stored at high temperature and RH

compared to other treatments. This is in line with the reports by van den Berghe (1990) who confirmed that fat becomes unstable at high temperature. The mechanism of hydrolysis could be that heat as well as water break down fat, to release keto acids which further undergo auto-oxidation with possible generation of rancidity products (Hamilton, 1989).

Supplementation of diets with inhibitor (antioxidant) resulted in a significant reduction in the rate of peroxidation. Many papers are in agreement with these findings (Waldroup *et al.* 1981; Berger, 1989). The mechanism by which vitamin E plays an antioxidative role is not fully understood but it may be attributable to the fact that it inactivates free radicals, products of the first phase of oxidation, or may reduce transition metals (Decker and Zhinin, 1998). In confirmation of the findings of Coppen (1989), inclusion of inhibitor did not decrease the rate of hydrolytic rancidity in diets. Diets with the inhibitor were observed to have higher concentration of FFA than those without the inhibitor.

The rate of peroxidation of fat in diets with the inhibitor increased after one month of storage, which was much more noticeable at a high temperature. This change may be due to the fact that the anti-oxidative strength of vitamin E, the antioxidant in the inhibitor, was reduced. Vitamin E also undergoes deteriorative changes similar to fat and other fat-soluble vitamins, with age (Paster *et al.*, 1985; Khalil and Mansour, 1998) or may be leached away (Lang, 1977), especially at high temperature and humidity. The rate of fat hydrolysis and peroxidation in this study was observed to be higher than that reported in the first experiment as confirmed by the concentrations of FFA and Pv. This may be due to a higher fat content of the diets used in this study than that of the diets

reported in chapter four (Njobeh, 1997; Ruiz *et al.*, 2000). Furthermore, the sources of fats in the diets used in the present study may contain a higher degree of unsaturation than the source used in the previous experiment (Sanders, 1989; Berger, 1989)

5.2.3 Feed microbiology

Microbiological analysis detected no toxins although *Aspergillus spp, Penicillium spp* as well as *Fusarium spp* were fungi identified in the diet prior to storage. Similar results were obtained in experiment one (Section 4.3.2). Although these fungi are the most predominant in South Africa (Devegowda *et al.*, 1998) and are found in maize-containing feeds (Dutton and Kinsey, 1996), it is likely that the toxins tested were below detection limits. Furthermore, the ingredients used in the formulation of the diets might have been fresh or well preserved to limit microbial growth and production of mycotoxins.

5.2.4 Production parameters

The influence of storage temperature, RH, inhibitor and toxin-binder on feed intake was less definitive. From the rancidity parameters, diets stored at high temperature and RH without inhibitor were found to be of lower quality (containing high FFA and peroxides) and thus were expected to be rejected by birds. With regards to feed intake, these diets were not different from those high in quality (low FFA and Pv). From these findings, it can be suggested that the concentration of FFA and Pv might not have attained the threshold level to induce loss of appetite and or feed refusal. Although microbiological studies were not conducted during storage, it is likely that storage at high temperature (30°C) and RH (80%), fungal growth and possible production of mycotoxins may have occurred. Furthermore, addition of a detoxifier in diets had no

observable effect on feed intake. However, such results are expected, as inclusion of a detoxifier in diets has no effect on feed quality.

A direct relationship between feed intake and body weight was not observed in this study. For example, an increase in feed intake was not accompanied by a uniform increase in body weight. Unlike feed intake, body weight was improved in birds fed on diets that were supposed to be high in quality. The lower body weights observed in birds raised on diets high in FFA and Pv might have been as a result of diarrhoea (observed in some birds), a common biological symptom of rancid feed (Shermer and Calabotta, 1985) or immuno-suppression from toxins (Raju and Devegowda, 2000). At high temperature, inclusion of the inhibitor improved the body weight of birds. Vitamin E prevents encephalomalacia (Lin *et al.*, 1989) although it was not determined if birds on the diets without the inhibitor had sub-clinical encephalomalacia during the trial.

Birds fed diets supplemented with the detoxifier were observed to have higher body weights when compared to the control. Many previous studies are in agreement with the findings of this study (Shane, 1999; Devegowda *et al.*, 2000; Raju and Devegowda, 2000). The mode of action of the detoxifier used in this study, like other sorbent materials may be mediated by effective absorption and efficient utilisation of nutrients by birds (van den Berghe, 1990; Raju and Devegowda, 2000). Various sorbent materials have been effectively used in controlling mycotoxins (Bailey *et al.*, 1998). The detoxifier may also bind toxins while in the GIT, to form complexes, thus preventing the toxins from being absorbed into the body (Raju and Devegowda, 2000).

Just as feed intake was not correlated to body weight in this study, neither was FCE. The lack of a definite pattern in feed intake was noticeable in the data on FCE. However, birds fed diets stored at low RH had higher FCE than that of those on diet stored at high RH, irrespective of storage temperature. Previous studies have demonstrated that the use of sorbent materials such as mycosorb, bentonite and clay were effective in reducing the deleterious effects of mycotoxins in broilers and other birds. Although supplementation of diets with the detoxifier (Mycosorb) did not significantly improve FCE, results indicate that birds offered diets with detoxifier had higher FCE when compared to the control.

5.2.5 Visceral organ weights

Results of this study showed an increase in the relative weight of most of the organs assessed in the group of birds fed diets stored under adverse conditions without inhibitor or detoxifier. Mycotoxins have been demonstrated to influence organ weight, a measure used to evaluate the occurrence of mycotoxicosis in birds (Bailey *et al.*, 1998; Kubena *et al.*, 1998). Liver weight increased at high temperature and for birds fed diets without inhibitor or detoxifier. Furthermore, inclusion of inhibitor or detoxifier markedly reduced liver weight. Toxins have been known to be carcinogenic, with liver and kidney the most targeted organs. Results obtained in this study are similar to those obtained in previous studies regarding the influence of mycotoxins on the relative organ weight (Bailey *et al.*, 1998; Raju and Devegowda, 2000).

In the present study, gizzard weight was reduced with inclusion of inhibitor or detoxifier in the diets. Proventriculus and spleen were unaffected by any of the factors. Although this study did not attempt to determine the incidence of mycotoxins in the test

diets, it is obvious considering the adverse storage conditions, that there were some toxins produced. Inclusion of inhibitor might have reduced microbial growth and possible production of mycotoxins that would otherwise have negatively affected the biological response of the birds.

5.2.6 Serum biochemistry

Serum biochemical analysis was conducted in order to confirm the occurrence of mycotoxicosis in the study. This is because of the hypothesis that mycotoxins interfere with protein synthesis (Bailey *et al.*, 1998; Kubena *et al.*, 1998). Results of serum biochemistry revealed significant differences in all the parameters measured as affected by individual and combined factors. Concentrations of serum phosphate as well as protein and albumin were increased as a result of supplementation of diets with the detoxifier. These results are similar to those obtained by Khajarern and Khajarern (1999) and Abdel-Wahhab *et al.* (2002). The high concentration of serum phosphate and protein upon addition of toxin-binder (detoxifier) might have been due to increased phosphorus absorption as well as protein synthesis (Kubena *et al.* 1998), which would have otherwise been affected by toxins.

5.2.7 Tibia ash

Bone porosity or a condition known as 'rubbery' bones is influenced by mycotoxins, especially ochratoxins (Raju and Devegowda, 2000). Although in this study, data obtained indicated no sign of rubbery bones, tibia ash content increased upon addition of a detoxifier as observed in the study reported in chapter four. Ochratoxin has been reported to interfere with bone formation through the destruction of vitamin D_3 that plays a prominent role in bone mineralisation (Huff *et al.*, 1977, cited by Raju and

Devegowda, 2000). The protective role of the detoxifier used in this study is uncertain but could be suggested that some of the mycotoxins so produced might have formed complexes with the detoxifier and in the GIT (site for toxins detoxification) and eliminated (Valarezo *et al.*, 1997).

5.3 Conclusions

The experiment showed that high rate of fat deterioration in high fat diets, which was further compounded by high temperature and relative humidity. However, addition of an antioxidant in the diets reduced the rate of deterioration by limiting the concentrations of peroxides. The detoxifier was effective in preventing mycotoxicosis and possibly alleviating the negative effects of rancid feeds thereby optimising broiler performance in this trial.

CHAPTER SIX

6.0 General Discussion and Conclusion

6.1 General Discussion

6.1.1 Effect of storage conditions on feed quality

Feed quality in general, is influenced by nutrient composition as well as storage environment. Studies reported in this thesis showed the influence of storage environmental conditions on feed quality. The efficacy of mold inhibitor and antioxidant in preventing fungal growth and rancidity, respectively, as well as the value of the detoxifier in diminishing the deleterious effects of mycotoxins in broilers was evident in this study.

Results obtained revealed that storage of diets at high temperature (30°C) after one month resulted in an increase in the DM content of the diets. This was followed by increases in the CP and fat contents as a result of the loss in moisture content of the diets. Low temperature storage resulted in a decrease in DM content due to absorption of moisture from the environment and a reduction in loss of moisture from the feeds. However, in the second month of storage, diets stored at low temperature (15°C) had higher DM content than those stored at high temperature. Reasons for this abnormal increase are not clearly understood and thus, unexplained but may be due to vaporization of lipids at high temperature. The decrease in the fat content observed in the second month of storage might have been as a result of rancidity or volatilization of fatty acids.

Increase in the concentrations of Fe and Zn after one month in experiment one can only be attributed to moisture loss, while a decrease in the concentration of these transition

metals may be due to loss through leaching (Lang, 1977), presence of antioxidants (Decker and Zhinin, 1998) or their involvement in oxidative reaction. Inclusion of the inhibitor in the diets did not prevent loss of nutrients in these studies.

Both experiments showed that oxidation and hydrolysis of fat in diets were not completely avoided, regardless of storage conditions. Similar to other reports (Berger, 1989; Coppen, 1989), storage at high temperature *per se*, resulted in an increase in both oxidative and hydrolytic rancidity with a possible loss in feed quality. Fats are known to be highly unstable when subjected to heat as has been observed by other researchers (Hamilton, 1989; Sanders, 1989; Ruiz *et al.*, 2000). A higher degree of oxidation and hydrolysis was observed in treatments of the second experiment than those of the first experiment due to higher fat content in the latter.

The efficacy of the inhibitor (antioxidant) in reducing the rate of auto-oxidation of fat as a measure in maintaining feed quality was well established in both experiments by significantly reducing Pv. The concentration of peroxides in diets supplemented with the inhibitor was much lower than that of diets devoid of the inhibitor. Many antioxidants have been widely used to prevent oxidation of fats with successful results. The mode of action of antioxidants in preventing oxidation may be that, they inactivate free radicals produced during the induction phase, or they may render transition metals less accessible for oxidation (Decker and Zhinin, 1998).

These studies support earlier conclusion (Coppen, 1989) that the presence of inhibitor (antioxidant) did not reduce the rate of hydrolysis of fats in diets. Instead, diets supplemented with the inhibitor experienced a significant increase in FFA level in

experiment two and in most of the diets in experiment one. The increase in the rate of hydrolysis in diets with the inhibitor is not fully understood. Similarly, fatty acids could be synthesized from free radical components of these organic acids. However, the FFA concentration in the diets is often overlooked because it is not of any toxicological significance as fats undergo enzymatic hydrolysis in the GIT.

Results obtained in both experiments showed little or no variation in the concentrations of FFA as well as Pv. Similarities in the results obtained in both experiments may be as a result of lack of drastic differences between fat levels in the diets. Furthermore, sunflower oilcake and Chilean fishmeal, contain fats that are highly susceptible to rancidity, were used in high proportions in experiment one, resulting in an increase in the rate of hydrolytic as well as oxidative rancidity.

Microbial analysis was conducted only on fresh diets used in both experiments. Although both diets varied in the type of ingredients used, results indicated that the fungi identified were identical. The similarities in the species might be due to the similarity in the major feed ingredients used in formulating the diets. These fungi are the most common species found in South Africa (Devegowda *et al.*, 1998). The absence of toxins in the diets might be due to the fact that not all of the toxins of pathological importance were tested. It might also be that the toxins tested were below detection limit or there was no extensive fungal growth in the diets. Microbial analysis during and after storage might have provided conclusive results, but unfortunately, this was not done.

6.1.2 Feed utilization by broiler chickens

In both experiments, feed consumption was not affected by storage conditions. Rancidity, as an indicator of quality of feeds did not appear to influence feed intake. It can be deduced that the concentrations of FFA as well as peroxides in the diets might be below the threshold level to effect loss of appetite or feed refusal. On the other hand, low body weights and FCE observed in birds fed diets stored at high temperature and RH clearly demonstrate the poor quality of such diets influenced by adverse storage conditions. It is highly likely that these diets contained toxins, as conditions were favourable for mold growth.

Results of the present research indicate that body weight and FCE was improved when diets were supplemented with the inhibitor in experiment one while in experiment two, there was no improvement in body weight or in FCE on diets with inhibitor when compared with the control. Supplementation of diets with the detoxifier resulted in an increase in body weight and FCE in both experiments. The basic mechanism of the detoxifier in preventing mycotoxicosis may be that the reaction in which toxins are bound to the sorbent material or the sequestration of mycotoxins in the GIT.

6.1.3 Visceral organ weight, serum biochemistry and tibia ash

The target sites for mycotoxins are the visceral organs (Kubena *et al.*, 1990). In experiment one, the relative weights of the organs confirmed that diets stored at high temperature and RH were of poor quality. The weight of the liver, proventriculus and gizzard weights of such birds was higher than those of birds fed other diets. However, experiment two did not clearly demonstrate this effect. Results also included increases in the relative weight of the organs when diets were stored at high temperatures.

However, supplementation of diets with inhibitor reduced liver and gizzard weights in both experiments, similar to results obtained by other researchers (Bailey *et al.*, 1998; Kubena *et al.*, 1998). Inclusion of the detoxifier in diets decreased the weight of liver, heart, gizzard as well as spleen in both experiments as reported in previous research. No differences were observed between the results in the two experiments, probably due to wide variation.

Reports on serum biochemistry as influenced by feed storage is scarce. However, such results, along with organ histology are often used in the diagnosis of mycotoxicosis (Kubena *et al.*, 1990). Results obtained in this study revealed that storage conditions had no influence on the parameters measured in experiment one, while in experiment two, total phosphate level decreased with increasing storage temperature. Furthermore, low storage RH was observed to increase serum protein as well as globulin in the second experiment, which might have been that feed storage at low temperature prevent mold growth and possible mycotoxins production which interfere with protein synthesis (van den Berghe *et al.*, 1990).

The supplemental inhibitor was effective in increasing serum protein and globulin in the first experiment while in experiment two, only serum albumin was increased upon addition of the inhibitor. Inclusion of the detoxifier was observed to be beneficial in increasing serum phosphorus, protein and albumin in both experiments. Similar results have been reported by previous workers (Khajarern and Khajarern, 1999; Abdel-Wahhab *et al.*, 2002). The increase in the concentration of the parameters due to the presence of the detoxifier is probably due to the increase in phosphorus absorption as well as protein synthesis (Kubena *et al.* 1998). In the experiments reported in this thesis,

there was no observable difference between the results obtained as influenced by dietary fat level. This may be due to little or no influence of dietary fat level on serum biochemistry, within the limitation of the parameters measured.

Reports on bone porosity (a condition of poor calcium and phosphorus depositions) as influenced by feed storage conditions are also rare. Bone porosity is influenced by ochratoxins (Raju and Devegowda, 2000). Results obtained in these experiments showed no indication of 'rubbery' bones. There was an indication that storage temperature and RH did not affect tibia ash. Furthermore, inclusion of the inhibitor had negative or no effect on tibia ash. This negative effect is unknown and less conclusive. However, addition of the detoxifier to the diets was observed to increase bone ash in both experiments. The detoxifier prevents ochratoxins from interfering with bone formation.

6.2 Conclusion

This research showed that high storage temperature and relative humidity significantly decreased feed quality and thus would reduce the shelf-lives specified for animal feeds. Furthermore, a combination of physical (ingredient limit in feed formulation), chemical (preservatives) and biological (absorbents) methods in management of feed contaminants as a useful tool in optimizing broiler performance has been clearly demonstrated in this study. It is recommended that feeds that are destined for storage should not contain more than 5% fats with high degree of saturation, else, the diet should contain an antioxidant. For future research, the scope of this study should be broadened to include evaluation of mycotoxicological changes in feeds during storage.

The effects of mycotoxins on tissues, particularly the liver and kidney could also be evaluated using biochemical and histological techniques.

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Appendices

Appendix I Chemical analysis

Protein

The samples were weighed, loaded into a rack and placed in position in the autoloader with blanks and standards. Information on the amount of nitrogen content in the sample was then printed out. The value of nitrogen recorded is multiplied by 6.25 to get the crude protein content of the diet.

Dry matter

The crucibles to be used for dry matter analysis were oven-dried overnight, removed, put in a desicator to cool and weighed. A sample was weighed in the crucible and oven-dried at 135°C for 4 hrs. This was immediately removed and put in a desicator to cool. The final weight was taken and dry matter (DM) percentage calculated as:

$$\text{DM \%} = \frac{\text{Weight of sample after drying}}{\text{Weight of fresh sample}} \text{ X } 100$$

Fat

Approximately 10 g of sample was weighed and put into the thimble and the mass recorded. This was then placed in an oven to dry for 1 hr. The Buchi fat beaker was weighed and recorded. The Buchi 810 Soxhlet Fat Extractor (Brinkmann Instrument, Inc., Westbury, NY, USA) was switched on 15 minutes before loading samples. The thimble was plugged with cotton wool and petroleum ether poured to the beaker to approximately three-quarters of the brim. The thimble was placed into the extractor and heated for about 4 hr. At the end of the extraction process, flow of reflux fat free solvent was diverted to be collected in a container placed at the back of the machine. The free-solvent fat flask was opened for the thimble and beaker to be removed, then allowed to dry overnight for the solvent to evaporate off. The beaker was dried in an oven at 90°C

$$\% \text{ Fat} = \frac{\text{Mass of fat}}{\text{Mass of sample}} \times 100$$

for 1 hr and allowed to cool in a desiccator. The weight was recorded. Fat was calculated as:

Crude Fibre

Approximately 500 mg of sample was weighed and put into a sintered glass crucible, which was lowered carefully into a modified beaker. Into the crucible, 75 ml of hot H_2SO_4 solution was slowly added and placed onto preheated heating unit to boil for exactly 30 min. Cold water was passed through condensers to facilitate reflux action. The beaker was removed from the heating unit while the crucible was carefully removed with the aid of a tong. The crucible was placed into a rubber adaptor in the proximal end of Buchner vacuum flask. The tap was then switched on and low vacuum used to suck H_2O to the condenser. After switching off the tap, boiling water was introduced into crucible and low vacuum reapplied (x3) to wash out all the H_2SO_4. The crucible was re-introduced into the beaker, 75 ml hot NaOH was added and then placed onto the heating unit to boil for exactly 30 min. The crucible was carefully lifted out and filtered as initially done with suction vacuum, then washed with boiling water. The crucible was dried overnight in an oven, cooled in a desiccator and weighed. Finally, it was placed in a furnace at 550°C for 3 hr, cooled and weighed. Crude fibre was then calculated as:

$$\% \text{ Crude fibre} = \frac{[(\text{crucible} + \text{dried residue}) - (\text{crucible} + \text{ash})]}{\text{Mass of fresh sample}} \times 100$$

To correct for the % fat and % moisture which were initially removed, the crude fibre (as is basis) was thus calculated as:

$$\% \text{ Fibre (as is)} = \frac{\% \text{ fibre x } (100 - \% \text{ fat} - \% \text{ moisture})}{100}$$

Ash

The weight of crucible and lid, which were initially oven-dried and cooled, was taken and while on the scale balance, approximately 1 g of sample was added. This was placed in a furnace set to about 550 °C and allowed to ash overnight. The crucible was removed from the furnace and placed in a desiccator to cool and weighed to record the mass. Calculation of ash was as follows:

$$\% \text{ Ash} = \frac{\text{Mass of ash}}{\text{Mass of sample}} \quad X \quad 100$$

Minerals

Sample preparations for calcium (Ca), phosphorus (P), zinc (Zn), iron (Fe) and copper (Cu) analyses were similar. The samples were dried and milled. Exactly 500 mg of sample was weighed into a conical flask and 18 ml of acid mix (1:4, HNO_4: $HClO_4$) was added. The solution was placed on a digestion block to digest until white fumes were observed and the solution cleared. The sample was removed from the block and allowed to cool. The mixture was then transferred to a 250 ml volumetric flask to make a 1:50 dilution to the mark with deionised water. The standards were prepared by using a certified standard solution of 1000ppm perchloric acid solution. The combined working standards for Cu, Fe and Zn were 1, 2 and 3 ppm, respectively while those for Ca and P were 100 and 50 ppm, respectively.

In analysing Fe, Cu and Zn, glasswares were prepared by soaking in 10% V/V HNO_3 and thoroughly rinsed with distilled H_2O. Five grams of the test sample was weighed into a 100 ml Pyrex beaker, evaporated to dryness and charred on a hot plate. The

beaker was transferred to a muffle furnace at a temperature of 450 °C and left overnight. The beaker was removed from the furnace and covered with a clean watch glass. It was later moistened with H_2O and evaporated to dryness to make sure all the carbon has been completely removed and ashed (x 2). The beaker was allowed to cool, 2 ml of HNO_3 and 2 ml of H_2O were added to the residue and warmed. The solution was transferred into a 250 ml volumetric flask and washed several times with warm H_2O. This was cooled, diluted to volume with H_2O and mixed thoroughly. A reagent blank was prepared by following the same procedure without addition of sample. The calibration solutions for particular metals were prepared by diluting the appropriate solutions with an equivalent concentration of HNO_3. For iron, copper and zinc, these were 0-5, 0-4 and 0-1µg/ml, respectively. Absorption was read at 248.3, 324.7 and 213.9 nm, respectively for iron, copper and zinc. The metal content was calculated as:

$$\% \text{ Metal content} = \frac{100\ V\ (P - B)}{M}$$

where P is mg/l of metal in sample solution aspirated, B is mg/l of metal in blank solution, M is weight of sample in g, and V the volume of sample solution in ml.

AME_n and TME_n

A rapid *in vivo* technique is used in which adult roosters are force-fed with a measured quantity of test sample following a 24 hr fasting period during which time, the birds were given glucose in water (50 g:50 ml) per bird to ensure that they do not become dehydrated or lacking in energy. After feeding, excreta were collected over the following 48 hrs, dried, weighed and analysed for energy to determine the excreta output and compared with the energy input to determine the digestibility of the energy consumed.

A correction was made for endogenous energy losses (EEL), as well as for nitrogen that may either have been retained or lost during the assay period. The resultant AME value does not include a correction for EEL, whereas the TME does. Both values were corrected for nitrogen balance. Gross energy was determined with a DDS isothermal CP500 bomb calorimeter (Digital Data Systems (Pty) Ltd., Randburg, RSA).

Calculations were done as follows.

$$AME_n/g = \frac{[(F_i \times GE_f) - (E \times GE_e)] - (NR \times K)}{F_i}$$

Where F_i is feed intake (g); E is the faecal output (g); Ge_f is the gross energy of feed; GE_e is the gross energy/g of excreta; $NR = (F_i \times N_f) - (E \times N_e)$; N_f is the nitrogen /g of feed (g); Ne is the nitrogen/g of excreta (g); and K is a constant (34.4 or 36.5 kJ).

$$TME_n/g = \frac{[(F_i \times GE_f) - (E \times GE_e) - (NR \times K)] + [(FE_m + UE_e) + NR_o \times K)]}{F_i}$$

where Fe_m and Ue_m are the energy of that portion of the faeces other than feed residues and the portion of the urinary energy not of direct feed origin, respectively. NR_0 is the nitrogen retention estimate of fasted bird and NR is that of fed birds.

Appendix II. Feed additives used

Name: Mold-Zap Powder

Use: Fungal inhibitor

Ingredients: Porpionic acid (Min. 25%), Ammonium hydroxide, Sorbic acid, Maize meal, Vermiculite and Neutral flavours.

Name: Banox E

Use: Antioxidant

Ingredients: BHT, BHA, Propyl gallate, Limestone and citric acid.

Name:MTB 100

Use: Detoxifier

Ingredients: Brewers dried yeast, Brewers fermentation solubles, Silicon dioxide and Calcium carbonate.

Appendix III. Microbial analysis

FUNGAL SCREEN

Each sample was weighed (1g) and suspended in Ringers solution (10ml). The sample suspension was serially diluted (1ml) in Ringers Solution to 10^{-4} diluted of the original concentration. Thereafter of each dilution (1ml) was transferred to sterile petri dishes. The agar that was used for the pour plates was Ohio Agricultural Experimental Station Agar (OAESA). The agar was prepared (250ml) and autoclaved (20min). After autoclaving the agar was allowed to cool. Thereafter Streptomycin (Sigma) and Chlorophenicol (Merck) solutions (2ml) were added. The agar was then poured (20ml) into each petri dish, the plates were gently swirled and the agar was allowed to set. The fungal plates were then incubated for seven days at 28^{0}C. After the incubation period the fungal plates were removed and the growth was noted. The fungal colonies were examined macroscopically and there features noted. The fungi were examined microscopically to identify the type of spores and mycelia present in the fungi.

A loop full fungal spore was suspended in a loop full of water and mounted on a sterile slide. The smear was allowed to air-dry which was followed by the addition of one drop

of Lacto phenol Blue. A cover slip was then gently placed on each slide. The slides were examined using at a 100x magnification.

Extraction of toxins

After weighing the samples acetonitrile (Merck)/4% potassium chloride solution (9:1v/v) (100ml) was added. The samples were shaken for one hour in a Controlled Environment Incubator Shaker (New Brunswick Scientific, USA). Thereafter the samples were removed and transferred to a Buchner flask and filtered through a Whatman No.1 filter paper on a Buchner apparatus (model Al 17). The solid material was discarded and the filtrate transferred to a 250ml-separating funnel. The filtrate was defatted twice with iso-octane (50ml) (Merck) and gently inverted funnel. Two distinctive layers were formed, the bottom layer was retained in a 250ml conical flask and the top fat containing layer was discarded. The extract was transferred back into the separating funnel and freshly prepared saturated sodium bicarbonate solution (Merck)/distilled water (50ml) (3:2v/v) and Dichloromethane (DCM)(50ml)(Merck) was added. Inverted flask gently to avoid emulsion, after a few minutes two distinctive layers were formed: DCM layer (Neutral Fraction) and the aqueous layer (Acid Fraction).

The Neutral Fraction was extracted with three portions of DCM (25ml), filtering each portion through a bed of sodium sulphate anhydrous (Merck), (10g) in a folded 11cm filter paper in a small funnel placed in the neck of a rotary evaporator flask. The rotary evaporator flask was inserted onto a Buchi Rotary Evaporator (Model 6540/2PR, Swiss) speed adjusted at 4, with a water bath not exceeding 60^0C. The residue obtained was dissolved with acetonitrile (2ml). A piece of previously soaked dialysis tubing (25mmx16mm) (Sigma)(25cm soaked in distilled water for one hour) was firmly tied on

one end to form a closed sac. The dissolved residue was transferred into the dialysis tube using a 2ml Pasteur pipette and a knot were tied at the top. The dialysis tube was placed in a boiler tube (15x25cm) containing acetone (Merck) (30%v/v) (50ml). The boiler tube was sealed and was placed on a shaking table overnight. After shaking the boiler tube was removed from the shaker. The dialysis tube was discarded and the dialysate was transferred into a 250ml-separating funnel. This was again extracted three times with DCM and then rotary evaporated the samples. The residue was redissolved with DCM and transferred into a 10ml screw cap vial.

The Acid Fraction was treated with 1M sulphuric acid (25ml) (Merck) solution, to neutralize the sodium bicarbonate and waited four hours until effervescence subsided. Extracted three times with DCM, filtered through a bed of sodium anhydrous and rotary evaporated. Residue was redissolved with DCM (2ml), and stored in 10ml screw cap vial.

The vials containing the acid and neutral extracts was placed on a heated hot plate (50^0C) and evaporated with a steam of dry nitrogen, after which it was used for the detection step.

THIN LAYER CHROMATOGRAPHY (TLC)-SEPARATION/ DETECTION

The Neutral and Acid extracts were resolubilised with of DCM (200µl). Each aluminum backed TLC plate (10 x 10cm) (pre-coated with silica gel 60G) (Merck) was faintly marked with a pencil to mark the origin. Thereafter each extract was spotted (20µl) onto the origin of the plate in portions (5µl) and drying at each stage by gently blowing on to the spot. The first solvent system used was CEI. Placed the CEI (20ml) in a TLC tank (12x12cm) and equilibrated (30 min). Each plate was inserted into the tanks placing the

origin in the bottom left hand corner. The solvent was allowed to run 1cm before the top of the plate. Removed plate and marked the solvent run. The plates was allowed to dry, and was then inserted at right angels to the first run, into the second solvent TEF system in a different tank.

The plates were then sprayed by propping it against a large glass plate, standing in a vertical position in a fume cup hood. CTA (Sigma) was used for the Neutral plates and PRA for the acid plates. The spray gun was held (50cm) away from the plates and completely sprayed the surface of the plates with the spray reagents. Neutral plates were viewed after heating (2 min at 110^0C) and Acid plates immediately after spraying. After spraying each plate the unknown metabolites was detected by its characteristic colored spots that was produced by the spray agents. Each spot was circled with a pencil and labeled according to the colors produced e.g. Purple (P). The concentration of each metabolite in the original sample was estimated by the intensity of the spot. The Retardation Factor (Rf) was calculated by using the following formula:

$$Rf = \frac{\text{Distance of solute}}{\text{Distance of Solvent}}$$

The Rf values were calculated for the CEI and TEF runs, and were compared against standards that were visible with the spray agents used.

PREPARATIVE TLC – ISOLATION OF THE METABOLITES

Washed each glass plate (20x20cm) with distilled water, cleaned with acetone (100%) (Merck) and dried. Weighed out silica gel 60G (60g) (Merck, Germany) into a 250ml beaker and added distilled water (70ml), mixed and added to Shandon Southern Unoplan Spreader (Shandon Southern Unoplan, USA). The slurry was than immediately

coated on the plates (0.3-mm thickness), which was than rested (10min), and baked (110^0C) in an oven overnight.

After baking the plates, it was removed and allowed to cool. Thereafter carefully drew a faint straight pencil line, above the plate (1.5cm) to mark the origin. Each sample was redissolved in DCM (600µl) and vortexed. The plates were then spotted with each sample (200µl), in portions (10µl). Each concentrated spot had an interval of 1cm.The TLC tanks (22 x22 cm) fitted with stainless steel lids were equilibrated (30 min) with TEF (100ml). The plates were then inserted plate and were left to run (2 hours). Plates were than removed and air-dried. A portion of each plate was sprayed with the detection agents mentioned in 2.4.4, by placing a glass plate on top of the silica plate leaving a portion exposed to the spray reagents (Fig 3.6). Detected the characteristic metabolites, which appeared as straight bands. The bands were than scored off the plates, omitting the sprayed portion. Each metabolite was eluted from the silica gel with DCM (500ml) in equal portions (5ml), and was filtered through a sintered funnel, placed on a rotary-evaporating flask. The eluent was than rotary evaporated. The residue was dissolved with DCM (2ml) and nitrogen dried. Each metabolite was respotted on a TLC plate (10x10cm) to confirm purification of the metabolite.

Appendix IV Rancidity

In addition, to obtain enough fat required for rancidity tests, about 20 g of feed sample was used for fat extraction. Fat extracted from the feed was then used in rancidity (peroxide value and free fatty acids) analyses.

Peroxide Value

Peroxide value was determined by weighing out 5 g of fat into a quickfit round bottom flask; into it was added 12 ml of chloroform which was gently swirled to dissolve the

fat. Then, a mixture of 18 ml of glacial acetic acid and 0.5 ml of saturated KI was added. The flask was stoppered and shaken vigorously for 1 minute and put in a dark cupboard for another minute. A magnetic stirrer bar was included with 30 ml H_2O, stoppered and shaken vigorously. If pale yellow coloration was seen, less than 30 ml H_2O was added. The stopper was then removed and the flask placed on a balance and tared to zero. While on a magnetic stirrer, 0.002N $Na_2S_2O_3$ was titrated until the colour was almost lost. The weight was taken and again tared to zero. Into the flask, 0.5 ml 1% starch was added while stirring and slowly titrated with same concentration of $Na_2S_2O_3$ until the blue colour disappeared. The mass of titrant was recorded. This procedure was repeated for the blank. The volume of titre was obtained by subtracting that of titrant (blank) from the combined sample titrations. The following was done:

$$Pv = \frac{M \times N}{m} \times 100$$

where M is mass of titrant (blank corrected), N is normality of titrant and m the mass of fat used. Pv is measured in milli-equivalent of O_2 per kg fat (mEq/ kg)

Free fatty acid

For the determination of free fatty acids, neutralized ethanol was prepared by adding 50 ml ethanol with 0.5 ml phenolphthalein into a quickfit round bottom flask and heated to boiling on a steam bath. This was neutralized with 0.1N KOH until the pink colour persisted for at least 15 seconds. Two grams of fat was weighed and put into a flask, along with a few silica beads, and 25 ml neutralized ethanol added and brought to boiling point on a steam bath. This was then titrated with 0.01 N KOH until a pink colour was observed. The weight was noted and the following calculation implicated.

$$\% \text{ FFA} = \frac{VcM}{10m}$$

Where V is volume of titrant, c is normality of titrant, M is the relative molecular mass of acid chosen for the expression of results (e.g. oleic acid = 282) and m is mass of the test portion.

Appendix V Serum biochemistry

Fifty µl samples, standards and blanks were pipetted into the tubes. Five ml of Biuret reagent was pipetted into the blank tubes and allowed to stand for 40 min, after which, the reagent was added to first the standards and then to the samples. These were allowed to stand for 40 min and then read on a UV-VIS spectrophotometer at 540 nm.

Serum albumin content was determined by bromocresol green (BCG) binding. Twenty µl of samples, standards and blanks (deionised water) were pipetted into the test tubes. Five ml of the buffered dye solution was pipetted into the blank tubes and allowed to stand for 30 min after which, the dye solution was added to first the standards and then to the samples. These were allowed to stand for 30 min and read on the UV-VIS spectrophotometer at 540 nm. Serum globulin is determined by calculating the difference between the serum protein and albumin values.

For serum phosphate analysis, 0.2 ml of sample was pipetted into the tubes for blank and 0.2 ml of the 100 ppm standard into another set of tubes. Into the tubes, 3 ml of Fe – TCA was added and centrifuged with sera samples at 2500 rpm for 10 min until precipitation occurred. Another set of clean test tubes were labelled and supernatants from the centrifuged samples were decanted into the new test tubes. Ammonium

molybdate solution, 0.5 ml was added to all the test tubes, allowed to stand for 10 min and then read on a UV-VIS spectrophotometer at 623 nm.

Appendix VI Rancidity graphs

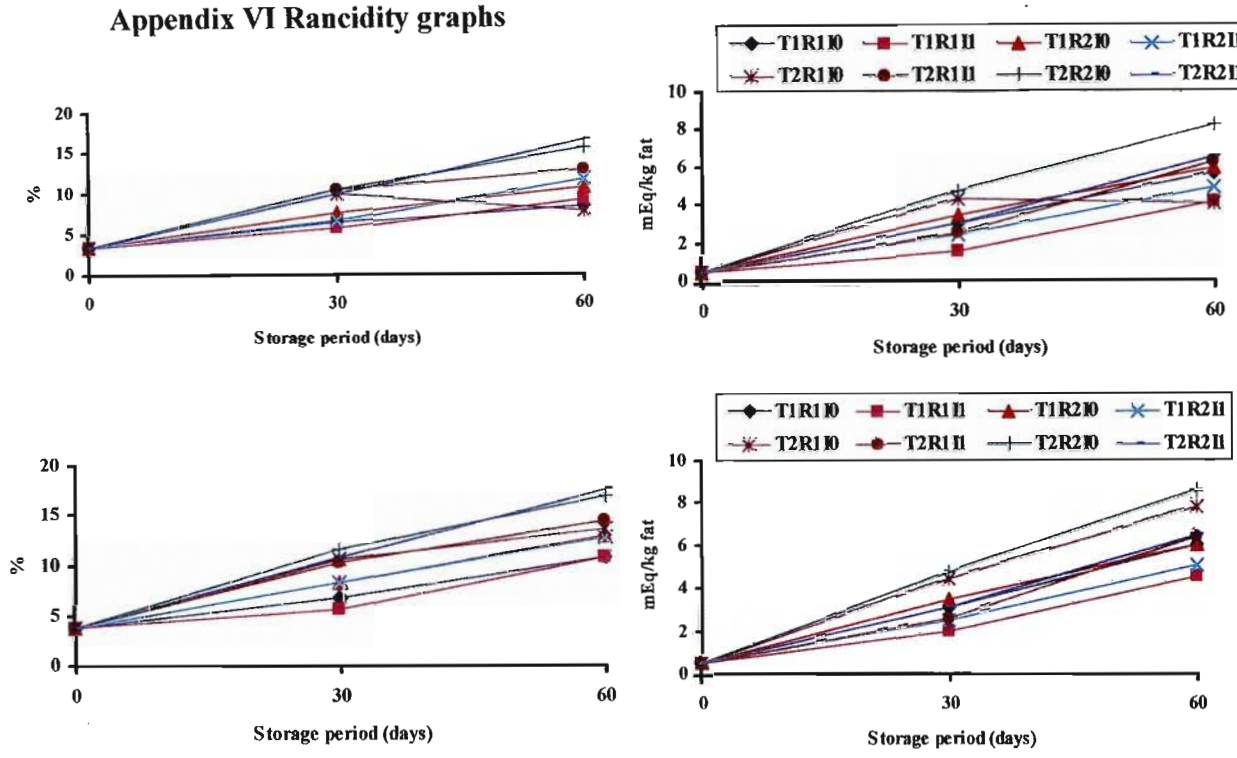

Figure A Changes in concentrations of free fatty acids (*left*) and peroxide value (*right*) with time in Experiment One (*above*) and Two (*below*). T1, temperature 15°C; T2, temperature 30°C; R1, RH 50%; R2, RH 80%; I0, without inhibitor; I1, with inhibitor. Samples were analysed in duplicates and mean values derived.