COMMON BEANS AS A PROTEIN SOURCE IN BROILER DIETS: EFFECTS OF PROCESSING, ENZYMES AND PROBIOTICS ON ANTI-NUTRITIVE FACTORS AND BROILER PERFORMANCE

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other university.

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REG. NO. KD11/0197/07

RECOMMENDATION

This thesis has been submitted with our recommendation and approval as University supervisors:

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DEDICATION

To my dear wife Brenda and loving children Kim, Evans and Joy, who are the joy of my life and my greatest achievement. My parents, Kiunga and Mwathimba who sacrificed everything to see that I got an education. And to the community who urged me on.

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To all I say God Bless You

ABSTRACT

A major challenge to Kenyan Livestock industry today is the inability to produce high quality feeds cheaply. Most of the common sources of protein such as soybean meal, cottonseed cake and fishmeal used in Kenyan feed industry are imported. This makes the final products expensive. To reduce costs, most manufacturers resort to using inferior quality alternatives that result in inferior feeds and leads to slow growing animals. Experiments were conducted to evaluate common beans as an alternative that may solve the twin problems of inferior quality feeds and expensive ingredient imports. The first experiment was designed to evaluate the chemical composition of various common bean varieties grown locally and the effect of processing on the ant-nutritive factors (ANFs) they contain. The results showed that local beans are high in CP (18 - 23%) which varies with variety. They are also high in ANFs. Lectins activity varies from 4 HU/mg to 16 HU/mg while Trypsin Inhibitor (TI) ranges from 2.13 TIU/mg to 3.04 TIU/mg. The results also indicated that soaking red haricot beans for 24 hours followed by cooking in boiling water for thirty (30) minutes is effective in eliminating ANFs while solid state fermentation is partially effective. The second experiment evaluated the effects of multienzyme supplementation on red haricot bean ANFs and utilization by broilers. The results indicated that enzymes have no effect on ANFs in red haricot beans. These results also indicated that oven heating had little or no effect on ANFs in red haricot beans. The third experiment evaluated the effects of cooked red haricot beans, multienzyme or probiotic supplementation on broiler performance. Diets based on boiled beans supported a broiler performance similar to maize–SBM control. Enzymes and probiotics had no beneficial effect on diet utilization. A final experiment was conducted to determine the appropriate level of boiled red haricot beans in broiler starter rations. The results showed that red haricot beans boiled for 30 minutes can be included in broiler starter rations at the rate of 20% in the diet (50% replacement of soybean protein). These results show there is potential in utilizing locally grown common beans in broiler starter diets.

Key words: **anti-nutritive factors, broilers, enzymes, probiotics, red haricot beans**

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LIST OF ABBREVIATIONS

ANF	Ant-nutritive factor
AME	Apparent Metabolizable Energy
CF	Crude Fibre
DM	Dry Matter
EM	Effective Microorganisms
EE	Ether Extracts
FCE	Feed Conversion Efficiency
FCR	Feed Conversion Ratio
GLP	Grain Legume Project
IDF	Insoluble Dietary Fibre
NFE	Nitrogen Free Extracts
NIR	Near Infra Red
NSP	Non Starch Polysaccharides
RSM	Rape Seed Meal
SBM	Soy bean meal
SDF	Soluble Dietary Fibre
TDF	Total Dietary Fibre
TIA	Trypsin Inhibitor Activity
TIU	Trypsin Inhibitor Unit

CHAPTER ONE
1. GENERAL INTRODUCTION

1.1 Background information

Today, the animal feed industry is under the spotlight in view of the reports linking several human health and environmental problems with the animal feeds (Pluske, 1998; Lyons, 1998; Klein-Hessling, 2002). Some of the highlighted issues include:

(i) The relationship between use of antibiotics in animal feeds and increased antibiotic resistance in humans. Use of antibiotic growth promoters has consequently been banned in the European Union and Japan (Hruby and Pierson, 2002; Feed international, 2005).

(ii) The origins of bovine spongiform encephalopathy (BSE) and its human equivalent Creutzfeldt-Jacob Disease (CJD) due to re-feeding of slaughterhouse by-products to ruminants. This has led the European Union and United States of America to ban the use of these by-rroducts in ruminant rations (Lyons, 1998).

(iii) The link between current feeding practices coupled with intensification of production and increased environmental pollution. Livestock production is steadily moving from grazing and mixed production systems to factory type intensive production systems (FAO, 1996). This is accompanied by production of massive quantities of animal wastes in small areas and consequent environmental pollution. It is estimated that total animal wastes in the USA is 130 times that from humans (Lyons, 1998). Animal pollution is mainly caused by mineral wastage, protein wastage, greenhouse gases and bad odors. Due to serious pollution problems from intensive livestock industries, countries such as Singapore have banned keeping of pigs (FAO, 1996) while in the USA legislation is increasingly being introduced to regulate setting up of new intensive livestock industries. Kenya is yet to suffer from this kind of pollution crisis for our intensive livestock industry is still at its infancy.

To address the above problems, the animal feed industry should now target production of feeds:

(i) With natural alternatives to antibiotics; less mineral, energy and protein wastage; and that leave no pathogens or other residues in animal products.

(ii) That addresses the sensitive issues of genetic engineering. Most consumers have not completely accepted animal products produced through genetic engineering.

(iii) That minimizes animal protein recycling.

This shift from the traditional feed additives coupled with the resistance to genetic engineering and the increased restriction on animal protein recycling, calls for alternatives that sustain or even improve on current production levels without risking the health of the consumers in addition to being ethical and environment friendly (Klein-Hessling, 2002). Most researchers and producers in the European Union are currently occupied with finding other feed constituents that might lift digestive health and performance in place of the banned alternatives (Pig International, 2004). Measures to reduce nutrient wastage in feaces will also minimize environmental pollution, reduce feeding cost and also promote use of non-traditional feed ingredients in animal diets.

Soybean meal and fishmeal are the principle sources of protein in monogastric diets all over the world. USA is the worlds' largest producer of soybeans (FAO, 1994) meaning that most of the world has to import this vital protein source. The high cost of imports coupled with increasing demand for animal proteins in the developing world means that alternative sources of protein are required for the developing countries to develop intensive livestock industries capable of meeting the rising demand. Use of animal by-products is not tenable in view of the prevailing world mood against refeeding animal wastes to animals (Lyons, 1998). Banning use of livestock products in feeds is therefore expected to increase the demand and cost of alternative protein sources. Lack of alternatives is likely to further impact negatively on the livestock industries in the developing countries.

For developing countries, grain legumes may be one such alternative. Sixty percent (60%) of the world grain legume production is from developing countries (FAO, 1994). These grain legumes are currently not much used in livestock diets due to the antinutritive factors and the accompanying processing expenses. This is despite the fact that they have been reported as potential substitutes for SBM because of their similarity in the amino acid and energy profile.

Developing strategies to normalize their use in livestock industry will reduce the need for expensive imports in addition to promoting growth of intensive livestock industry. Increasing the use of grain legumes in poultry and pig feeds may therefore give economic benefits to these low-income countries in addition to spurring growth of these industries to meet the rising animal protein demand. This calls for development of cheap, efficient and easy to use processing techniques.

Vegetable proteins are inferior to animal proteins in their supply of essential amino acids. The problem is magnified by the presence of ANF's in most vegetable proteins that might result in unavailability of some of the essential amino acids present. Identification of the most limiting amino acids and supplying them from synthetic amino acids could greatly improve the value of plant protein supplements in addition to reducing their antinutritive effects. With the gathering international pressure against use of meat and bone meal in animal diets, the need for more information on use of vegetable protein is therefore even more urgent. Development of appropriate biotechnologies that improve vegetable protein utilization will cut down the cost of production, reduce nutrient wastage and cut on pollutants emanating from livestock units. Adopting use of legume proteins in feed industry will also spur growth of intensive livestock industry in Kenya. Future livestock production is predicted to be more intensive and crop based rather than extensive (FAO, 1996).

Kenya is the largest producer of common beans in Africa at 535,000 tonnes followed by Uganda (435,000 tonnes). In Kenya, common beans *(Phaseolus vulgaris*) are found in most regions of the country. The beans are hardly used in the feed industry mainly due to ANF's present and lack of appropriate easy to use processing techniques. Information on value of common beans as a feed is also lacking. Research done elsewhere advocates use of locally available grain legumes in those countries suffering a shortage of appropriate oil seed cakes (Dixon and Hosking 1992; Wiryawan, 1997). However research is required to document the types and quantities of ANF's present in local legume grain varieties and appropriate treatment methods identified before they are recommended for the feed industry. Much of the available data and information on the nutrient and anti-nutritive composition of the feeds do not cover all the feeds and where available, needs updating. This is because of the possible effects of variety/genetic origin, climate, soil, processing methods, pesticides and fertilizers on the chemical composition of the feed plants (FAO, 1966).

Poultry industry is the fastest growing sector of livestock production in Kenya today at the rate of 6% (Kabuage, 2002). The present poultry population is about 29 million birds consisting of 21.7 million indigenous and 7.3 million exotic birds (MARD, 1998). Emerging factors such as urbanization and rising consumer incomes are expected to fuel growth of the poultry industry especially in urban and peri-urban areas. Land fragmentation in high potential areas coupled with rapid population growth is also creating ideal conditions for further growth of the poultry industry in the rural areas of Kenya. To facilitate and aid in this growth, it is necessary to produce less expensive, high quality feeds. Alternative ways of enabling farmers to use locally available raw materials to compound simple rations should also be sought to improve the nutrition of the majority indigenous flocks. Indigenous birds which make up 70% of the population are generally poorly managed and are characterized by low productivity of both meat and eggs. The fewer exotic birds are managed commercially and contribute 55% of the eggs and 40% of poultry meat produced in the Kenyan market (MARD, 1998). The average productivity of our commercial flocks is however lower than international standards. Layers produce an average of 240 eggs per year while broilers take 6-8 weeks to attain 2 kg. International standards are 300 eggs per year and 2 kg in six weeks. In addition to low productivity of birds, the profit margins achieved by farmers are low forcing some to shift to alternative better paying enterprises (Kabuage, 2002).

The main reason advanced for low productivity and poor profit margins is the expensive and low quality feed produced by our local feed millers. Part of the reasons for costly and poor quality feed is the lack of appropriate locally available sources of protein and failure to adopt new biotechnologies in feed manufacture. Most of the protein rich ingredients are imported from neighbouring countries mainly due to failure of the country to develop a successful oil crop industry that would yield the required oil seed cakes. Poor marketing environment is partially to blame for most farmers abandoning oil crop farming in the country (Kabuage, 1996). The deficit is filled by imports from neighbouring countries. Small milling companies that lack resources to import ingredients usually rely on poor quality byproducts leading to variable quality feeds.

1.2 Feed quality and anti-nutritive factors

Of the total animal feed manufactured locally, 70% is consumed by the poultry industry (Kabuage, 1996). This implies that most manufacturers mainly produce poultry feeds. The main ingredients used are maize, wheat and their by-products as sources of energy; oil seed cakes, fish and meat meals as sources of protein. Since the country has a readily available energy source in form of maize, wheat or their by-products, the main problem area is in vegetable proteins. Legumes are being proposed as a suitable protein source in countries that lack sufficient quantities of oil seed cakes (Dixon and Hosking, 1992; FAO, 1996). However it is not much used in livestock diets due to the ANFs and the accompanying processing expenses. Establishing alternative easy to use and possibly cheaper processing techniques might increase use of this easily available legume in local animal feeds.

Kenya lacks a suitable protein source for use in the feed industry despite the fact that it produces large quantities of grain legumes. Common beans are the most popular legume variety and are grown in most parts of the country for human consumption. The feed industry has avoided using this legume partly due to its high cost, lack of guidelines on usage and tedious methods involved in removing the ANF's. Traditionally, legumes have been heated to destroy antinutritive factors and improve digestion (Arija et al., 2006). In oil cakes, the antinutritive factors are destroyed in the process of oil extraction and the resultant temperature rise. However, the resultant increase in temperature can lead to protein denaturation and decreased amino acid digestibility (Sefton, 1998). For legumes like *P. vulgaris*, heating also would increase the cost of feed production in addition to protein denaturation. Information on types and quantities of ANFs present, alternative methods of processing and inclusion levels for efficient utilization is lacking.

Since technologies such as use of antibiotics, hormones and products of genetic engineering are facing resistance from consumers, it may not be advisable to recommend use of the same for the local industry. Use of enzymes, probiotics and supplementation with methionine have been suggested as possible alternative methods of inactivating/reducing the ANF's, increasing digestibility of vegetable proteins and improving gut health (Wiryawan, 1997; Klein–Hessling, 2002). These substances are purchased off the counter and can be added as feed additives during the process of feed manufacture. If it can be shown that these substances can replace heating as a processing method and if they are cost effective to use, then routine use of common beans in poultry diets may be achieved.

Probiotics are selected and concentrated counts of lactic acid bacteria (lactobacillus, streptococcus) which are being promoted as alternatives to antibiotics (Klein-Hassling, 2002). They promote growth of beneficial microbes and eliminate harmful ones unlike antibiotics that eliminate all. The beneficial microbes also secret enzymes that may assist in degradation of indigestible feed materials (Jin et al., 1996a). It follows therefore that enzymes and probiotics might actually have similar effects on feed utilization and animal performance.

In the last 10 years, glucanase and pentosanase enzymes are routinely added to wheat and barley-based diets to reduce the antinutritive problems caused by glucans in barley and pentosans in wheat (Chalton, 1996). Phytase enzyme is also available commercially and is used to improve the utilization of phytate phosphorous in cereal grains. Whilst there has been widespread acceptance of cereal degrading enzymes in monogastric feeds, relatively less attention has been paid to identifying enzymes targeting ANF's occurring in vegetable proteins (Pluske, 1998). Reduced use of animal proteins and antibiotics coupled with expected increased reliance on vegetable proteins makes it even more urgent to identify appropriate enzymes to reduce ANF's in given vegetable proteins. Enzymes also are reported to reduce microbes in the GIT through increased digestion and absorption of nutrients. Hence they can be used in the absence of antibiotics to maintain production (Bedford, 2001).

Since the ANF content of common beans is known to vary widely (Dixon and Hosking, 1992; Cevdet and Gokgoz, 2007) it is important to document the ANF content of the available local cultivars. It is most important also for protein poor countries like Kenya to define more precisely the effects, if any, on various aspects of animal productivity of the antinutritive factors known to be present in many locally available high yielding grain legumes. Since vegetable diets are inferior to animal proteins in their supply of essential amino acids; presence of ANF's further lower their utility to monogastrics due to reduced nutrient digestibility, increased amino acid loss and poor gut health. Supplying the most limiting amino acids from synthetic sources could therefore greatly improve the value of vegetable proteins in addition to reducing the antinutritive effects.

The main objective of this study therefore was to investigate appropriate bio-technology treatments that would substantially enhance the use of the raw and processed common beans (*Phaseolus vulgaris*) as a major plant protein source in broiler chicken diets.

To achieve this, the following specific objectives were formulated:

1) To determine the chemical composition of the common bean cultivars (be consistent – are they cultivars or varieties?) in Kenya and establish the types and content of anti-nutritive factors.

2) To assess the effect of enzymes and probiotics on common bean ANF's and utilization of broiler chicken diets based on raw and processed common beans.

3) To assess the effects of cooking and fermentation on common bean ANF's and utilization by broilers and establish optimum inclusion levels in diets

4) To test whether enzymes and probiotics have similar effects on common bean ANF's as heating and fermentation.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Legumes in animal feed industry

Soybean meal and animal proteins such as fish meal, and meat and bone meal are the commonly used sources of dietary protein in poultry feed formulations around the world. In recent years, however, availability and the increasing cost of these ingredients are becoming serious threats to the continued expansion of the poultry industry. As a result, it has become necessary to evaluate alternative protein sources which can fully or partially substitute the conventional protein sources in poultry feed formulation. Grain legumes, such as common beans (*Phaseolus vulgaris*), are widely available in many parts of the world. These legumes play an important role as protein sources in both human and animal nutrition. However, their use in the poultry feed industry remains limited because of the uncertainty over their nutritional value and the presence of anti-nutritive factors which interfere with nutrient utilization resulting in poor animal performance.

In general, grain legumes are moderate to good sources of protein, containing 22 to 40% crude protein (Boulter, 1980; Hedley, 2001). The predominant protein fraction in legume seeds is made of globulins (60 – 90 %), which are storage proteins rich in arginine, glutamic acid, aspartic acid and their amides. However, legume seeds are deficient in sulphur-containing amino acids (Wang et al., 2003). The deficiency of these amino acids, however, does not pose a problem in commercial feed manufacturing because of the availability and low cost of crystalline methionine. The deficiency of methionine and cystine could also be overcome, in part, by mixing the legume seeds with cereal proteins such as maize gluten (Shewry and Tatham, 1999).

The importance of grain legumes either processed or unprocessed in diets for poultry and pigs have been extensively researched elsewhere and considerable information is available on their nutritive value. Effects of various processing methods and feed additives on the legume antinutritive factors have also been extensively researched and reported. The aim of this chapter is to review the published data on nutritional and anti-nutritional composition and the effects of various processing methods, feed additives, ANF's and utilization. The focus of the review will be on the common beans (*Phaseolus vulgaris*).

2.2 Nutrient composition of legumes

The protein content of grain legumes is between 20 – 42% and varies between and within the cultivars (Dixon and Hosking, 1992; Arija et al., 2006). The amino acid content is also dependent on environment and cultivar (Wiryawan, 1997). The table below demonstrates some of these variations.

Table 2.1 Proximate composition (g/kg) of some grain legume seeds or meal

	CP	EE	CF	ASH	NFE	Ca	P
Black gram seeds	260	15	50	40	635	2.0	4.0
Common bean seeds	240	20	40	40	660	2.5	4.0
Chick Pea seeds	220	40	90	30	620	1.0	4.0
Cow Pea seeds	250	15	40	30	665	1.0	4.0
Faba bean seeds	230	20	70	40	640	1.0	3.0
Field Pea seeds	250	15	60	40	635	1.0	3.0
Green Gram sees	240	15	50	40	635	2.0	4.0
Lentil seeds	250	30	20	40	660	1.0	3.0
Lupin seeds	322	60	145	31	442	2.2	3.3
Pigeon Pea seeds	200	20	80	40	660	1.5	3.0
Winged Bean seeds	380	150	120	40	310	3.0	3.0
Soya bean meal	498	7	53	71	371	2.5	8.6

Source: Ravindran and Blair (1992).

Generally all grain legumes can meet the amino acid requirements of non-ruminants as given in NRC 1994 except for methionine and cystine which are deficient. So if the diets are supplemented with the methionine and ANF removed, then the grain legumes are potential substitutes for SBM in monogastric diets. Metabolizable Energy (ME) value of grain legumes for pigs has been reported to be higher than for chicks. This has been explained to be due to the fact that pigs may be more able to use products of fermentation in the hindgut as sources of energy more efficiently than poultry. The ME values for legumes range from 8 MJ/kg – 13.8 MJ/kg and 13.5 MJ/kg to 15.87 MJ/kg for poultry and pigs respectively. Navy beans have an Apparent Metabolizable Energy (AME) value of 9.75 for chicken and digestible energy (DE) of 14.2 for pigs (Wiryawan, 1997).

2.3 Effect of feeding raw legume grains to poultry

Raw legume grains fed to monogastrics generally result in reduced growth rates, enlarged pancreas, and decreased fat absorption and lowered feed conversion efficiencies as compared with feeding processed legume grains such as soybean meal (Arija et al., 2006). The extent of the negative effects depends on the type of legume, the levels of inclusion and the age of the birds (Wiryawan, 1997). Different cultivars of the same legume vary in their chemical composition and hence produce different responses in apparent metabolizable energy (AME) and protein digestibility. Grant et al. (1991) reported different lectin levels in three varieties of *P. vulgaris* grown in Puerto Rico. However, all the three varieties were toxic to rats. When the seeds provided 50% of the dietary protein the rats had a low food intake and perfomed poorly while at 100% the rats lost weight and many died within 10 days of the experimental period.

Table 2.2 Quantities of ANF's in some grain legumes (g/kg air dry weight)

Legume	CF	ADF	NDF	Tannins	TI*
Black gram	43	96.3	148.4	16.22	5.44
Chickpea CV Desi	99	159.1	234.2	0.62	5.32
Chickpea CV kaniva	36.7	61.9	108.5	0.51	4.45
Faba bean	87.5	150.3	209.6	2.53	2.52
Field pea	57.8	98.5	177.6	0.61	2.57
Green gram	43.5	92.9	139.9	2.10	3.68
Lupin	47.0	203.7	239.4	1.53	<1
Pigeon pea	74.7	140.2	195.7	2.64	3.33
SBM	53.5	147.8	164.9	0.49	2.51

* TI (Trypsin Inhibitor) measured in TIU/mg

Source: Wiryawan, 1997.

Special processing techniques such as heating or oil extraction have to be employed to reduce the ANF's and maximize nutritional value to monogastrics. Ruminants with the help of microbes in the rumen can utilize raw legumes effectively without processing.

2.4 Common bean and its potential for use in animal feed industry

The common dry bean or *Phaseolus vulgaris* is the most important food legume for direct consumption in the world. Among major food crops, it has one of the highest levels of variation in growth habit, seed characteristics (size, shape, and colour), maturity, and adaptation. It also has a tremendous variability (> 40,000 varieties). Dry beans have numerous seed types, a wide spectrum of colours and colour patterns, varying degrees of brilliance and several seed shapes and classes. Of about 600 varieties grown in the world, 62 are commercial market classes and 15 of these are internationally recognized. The United States classifies dry beans as follows: Red Mexican, Pinto, Navy, Small White, Yellow Eye, Great Northern, White Marrow, White Kidney, Cranberry, Dark and Light Red Kidney, Pink and Black. These classes have become international.

Phaseolus vulgaris is produced in a range of crop systems and environments in regions as diverse as Latin America, Africa, the Middle East, China, Europe, the United States, and Canada. In Latin America, Africa, and Asia, the bean is primarily a small-scale crop grown with few purchased inputs, subject to biological, edaphic, and climatic problems. Beans from these regions are notoriously low in yield, when compared to the average yields in the temperate regions of North America and Europe (FAO, 2008). Yet yields can be improved in all zones with improved management eg?. The leading bean producer and consumer is Latin America, where beans are a traditional and significant food, especially in Brazil, Mexico, the Andean Zone, Central America, and the Caribbean. In Africa, beans are grown mainly for subsistence, where the Great Lakes region has the highest per capita consumption in the world. Beans are a major source of dietary protein in Kenya, Tanzania, Malawi, Uganda, and Zambia. In Asia, dry beans are generally less important than other legumes, but exports are increasing from China (FAO, 2008*)*.

Over 12 million tons of dry beans are produced annually world-wide; with a total production value of US $5717 million. Of this production, 81% occurs in tropical countries. Today, Brazil remains the most important country for production and consumption of beans in the world, followed by Mexico. The common bean (*Phaseolus vulgari*s), probably the most important grain legume in Africa, is grown on an estimated 3.7 million ha every year and provides food and income to at least 100 million people in eastern, central, and Southern Africa (PABRA, 2005). In this region, the bean is a major staple, providing the second most important source of human dietary protein and the third most important source of calories (Pachico, 1993). Per capita consumption in this region is perhaps the highest in the world, exceeding 50 kg in eastern and southern Africa and reaching over 66 kg in the densely populated Kisii districts in Kenya (Jaetzold and Schmidt, 1983). Kenya is the largest African producer (535,000 tonnes per anum) followed by Uganda at 435,000 tonnes (FAO, 2008).

Throughout sub-Saharan Africa mostly women farmers grow it traditionally as a subsistence crop. Yet the East Africa Bean Research Network's (EABRN) recent economic surveys show that approximately 50 percent of producers sell part of their harvest, primarily to urban populations. The income-generating aspect of bean production is becoming more significant principally near urban markets, where populations increasingly rely on beans as an inexpensive source of protein. Increased demand from feed manufacturers would further strengthen the commercialization aspect of common means and further contribute directly to women development and economic empowerment as most of the beans are produced by rural women. There are some limitations to the use of dry beans and research is finding ways to overcome them. The long preparation time can be inconvenient and expend much fuel. Anti-nutritive factors such as protease inhibitors and lectins can block the digestion process. Factors promoting flatulence are another undesirable effect. There is genetic variability for most of these factors.

The anti-nutrititive factors (ANF's) may be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992). Some of these chemicals are known as ‘‘secondary metabolites’’ and they have been shown to be highly biologically active. They include saponins, tannins, flavonoids, alkaloids, trypsin (protease) inhibitors, oxalates, phytates, haemagluttinins (lectins), cyanogenic glycosides, cardiac glycosides, coumarins and gossypol. Being an ANF is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal (Kumar, 1992). For example, trypsin inhibitors, which are ANFs for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke and Shull, 1985). The utility of the leaves, seeds and edible twigs of shrubs and trees as animal feed is limited by the presence of ANFs (Kumar, 1992).

2.5 Anti-nutritive factors in *P. vulgaris*

Most grain legumes contain secondary metabolites, which are resistant to gastric and intestinal digestion. These metabolites referred to as antinutritive factors have a protective role in plants and seeds but often have detrimental effects on mammalian digestion and metabolism (Dixon & Hosking, 1992). The main ant nutritive factors in *P. vulgaris* are lectins, Protease inhibitors, tannins and non-starch polysaccharides (NSP). Cevdet and Gokgoz (2007) have reported an average of 1.15% phytic acid, 0.56% total phenols, 0.06% tannins, 3374.74 TUI/g trypsin inhibitor activity and 70.74% protein digestibility.

2.5.1 Lectins (Hemagglutinins)

These are proteins that have the ability to bind glycoproteins and carbohydrates. They cause the clumping or agglutination of red blood cells in vitro. They also have a high affinity for sugar molecules. They have their principle antinutritive effect in the small intestines where they interfere with the absorption of the end products of digestion through binding to and disrupting the epithelial cells (Jaffe, 1980; Puztai, 1989). Lectins transported into the systemic circulation have been shown to result in impaired immunity and increased susceptibility to bacterial infection (Cheeke and Shull, 1985). Domingo (1990) reported a lectin activity of 167 in navy beans, 21 in cowpeas, 23 in lablab, and 14 in lupin grain measured as haemaglutination activity per milligramme dry matter of seed. Toxicity from various types of lectins has been reported to vary while nutrition performance is not always proportional to the lectin content (Grant et al., 1991). Using rabbit blood cells, Grant et al. (1991), reported haemagglutination activity of 4.2, 2.6, and 8.3 for *P. vulgaris* varieties Rosihna, G2, Carioca 80 and processor respectively. The same varieties had haemagglutination activity of 5.1, 2.6 and 4.2 for varieties Rosinha, G2, Carioca 80 and Processor respectively when tested on cattle blood cells. Lectins from all the tested *P. vulgaris* varieties were toxic to rats (Grant et al., 1991). Lectins are mainly destroyed through moist heating (Cheeke and Shull, 1985).

2.5.2 Protease inhibitors

Protease inhibitors interfere with protein digestion through binding tightly and specifically to the active sites of protein-hydrolyzing enzymes such as trypsin and chymotrypsin. They form stable, inactive complexes that are excreted with feaces. This increased excretion of proteolytic pancreatic enzymes is accompanied by compensatory hypertrophy of the pancreas. 50 – 100% increase in the size of the pancreas occurs in the presence of protein inhibitors (Leeson and Summers, 1997; Arija et al., 2006). Binding of the proteolytic enzymes reduces protein digestion and amino acid absorption from the small intestines (Birk, 1989). Sulphur containing amino acids of grain legumes are present largely as constituents of the protease inhibitors; for example 30-40% of the cystine present in common beans are constituents of protease inhibitors. Therefore since the protease inhibitors are resistant to small intestinal digestion and also bind to and result in increased feacal excretion of proteases (which are also rich in sulphur containing amino acids), availability of sulphur containing amino acids in grain legumes is likely to be low. The protease inhibitors may therefore induce secondary sulphur amino acid deficiencies. A trypsin inhibitor activity, measured as units of trypsin inhibited (TIU/mg DM) has been reported to be 3.22 in common beans, 2.59 in cowpeas, 1.58 in lablab and 0.95 in lupin grains. Protease inhibitors in beans are destroyed or reduced by heat (Cevdet and Gokgoz, 2007), fermentation or germinating for 48 hours (Leeson and Summers, 1997).

2.5.3 Nonstarch polysaccharides (NSP)

NSP are a large variety of polysaccharide molecules resistant to digestion mammalian endogenous enzymes. Polysaccharides that are not starches are poorly utilized by monogastrics because they have glycosidic bonds different from α – (1-4), (1-6) bonds that occur in starch. The NSP mainly occur in the cell walls and consists of water insoluble cellulose and water-soluble gums, hemicelluloses, pectic substances, and mucilages (Selvendran, 1984; Bjergegaard et al., 1997). The building blocks of NSP are pentoses (arabinose and xylose), hexoses (glucose, galactose and mannose), 6-deoxyhexoses (rhamnose and fucose), and uronic acids (glucuronic acid and galacturonic acids) (Pluske et al., 2001). NSP negatively affect digestion and absorption of starch, proteins and lipids in addition to delayed gastric emptying, increased intestinal transit time and increased pancreatic secretion (Nyachoti et al., 1997; Pluske et al., 2001). NSP in the cell wall also physically trapped starch and proteins and prevent enzymes from breaking them down.

Grain legumes contain high concentration of NSP, which are resistant to endogenous enzymatic digestion in the alimentary tract. Pigs to some extent digest NSP using microbial populations in the large intestines into volatile fatty acids which are absorbed to supply some energy. However, due to the short length of the large intestines and limited absorptive ability, little benefit is derived from bacterial digestion. On the other hand microbes in a fermentation tank decompose NSP into lactic acid which yields more energy for pigs than conversion of NSP into VFA's in the pig's large intestines (Pig International, 2004). Fermenting feeds containing high NSP content with lactic acid bacteria could therefore be a valuable way of breaking them down. Heating, dehulling or supplemental microbial enzymes targeting NSP are other methods used to reduce the effects of NSP.

2.5.4 Tannins

These are water-soluble phenolic compounds widely distributed in the plant kingdom with high protein binding capacity (Castanon and Marquardt, 1989). Most of the tannins occur in the seed coat. Tannins reduce protein digestibility and amino acid availabilities in pigs and poultry. Supplementation of feed with tanase and other normal digestive enzymes are likely to improve the protein value of tannin-containing grain legumes (Wiryawan, 1997).

2.6 Processes used to reduce/destroy the anti-nutritive factors

The effects of ANF are mainly reduced through mechanical treatments, water treatment, and heating. Enzymes, probiotics, fermentation and methionine supplementation are also being recommended as capable of removing/reducing the effects of ANFs.

2.6.1 Mechanical treatment (Dehulling)

Non Starch Polysaccharides (NSP) and tannins mainly occur in the testa (Selvendran et al, 1987). Removal of the testa through dehulling has successfully been used to reduce their effects and improve nutritive value of grain legumes. Weight gain, feed to gain ratio, apparent protein digestibility and apparent metabolizable energy were improved by 5.7, 7.0, 16.4 & 13.9% respectively when growing chickens were fed dehulled – high tannin peas compared with those fed untreated pea diet (Brenes et al., 1993b). This improvement could have occurred not only due to reduction of tannins but also due to reduction of fibre content associated with removal of the testa. Because the lectins and protease inhibitors are more concentrated in the cotyledons than in the hulls then dehulling is not an effective method for reducing lectins and protease inhibitors. Other methods need to be applied to extract them from the cotyledons.

2.6.2 Soaking

Soaking has been reported to improve the nutritive value of feeds for poultry (Shimellis and Rakshit, 2007). Adams and Nober (1969) reported that soaking barley grains in water for between 16-24 hours and drying before incorporation in diets elicitated an 8% increase in chick weight of four weeks. Similar effects have been reported in wheat and maize-based diets. The effect caused by soaking was attributed to rupture of the cell wall which then released the cell contents, mainly starch and protein to the digestive processes (Hesselman and Aman, 1985). Interest in water treatment as a means of improving nutritive value of feedstuffs has however waned off due to prohibitive energy costs of drying wet grain and the difficulties of giving feed in slurry, as a wet mash or meal (Mcnab, 1992)

2.6.3 Heat treatment

Heat treatment is the most widely used method of destroying the ANF (Wiryawan, 1997; Cevdet and Gokgoz, 2007; Shimelis and Rakshit, 2007). Most of the anti-nutritive components present in legumes are removed during classical cooking treatments (Abbas et al., 1987). Several different heat treatments can be applied to reduce mainly the heat – labile protease inhibitor, lectin activity and NSP. These heat treatments are autoclaving, irradiation, extrusion, toasting and boiling.

It has been shown that the pectins are partially solubilized during cooking of dry beans. Indeed, light microscopy gave evidence of the disappearance of middle lamella separating the cells, after the cooking treatment (Noah et al., 1998). Legumes, which are rich in starch and fibers, are of particular interest because they often contain starch resistant to digestion (Noah et al., 1998). In this study all the resistant starch was present in an "encapsulated" form and the fact that the authors could not observe any free starch granules outside the cells suggested that the cell wall may have played a role in protecting starch from digestion through impeding the access of digestive enzymes. The cell walls have been shown in vitro to greatly decrease the accessibility of enzymes to starch (Englyst et al., 1986, Tovar et al., 1992). Treatments that disrupt the cell wall such as heating and fermentation would therefore lead to increased starch digestion.

Dry heating is less effective than steam heating, autoclaving and extrusion (Huisman and Tolman, 1992). The authors reported that dry roasting inactivated 54-82% of the protease inhibitors while extrusion and autoclaving at 100^0C for more than 15 minutes inactivated 78-98% and 85-100% respectively. A recommended method is to boil the beans for at least ten minutes. Choice of the method to use however depends on availability of facilities and the economic considerations (Wiryawan, 1997).

It is important to find the exact conditions of heating which will maximize the destruction of the ANFs while minimizing damage to the feed protein. Excessive heating reduces protein solubility and may destroy certain amino acids. In the event that excessive heating occurs free amino groups of proteins react with reducing sugars such as glucose to form complexes that are not hydrolyzed by digestive enzymes. Such carbohydrate-amino acid reactions referred to as maillard or browning reactions, mainly affect lysine (Scott et al., 1982). Arginine, histidine and tryptophan are also affected. Navy beans presoaked overnight before cooking lost all their lectin activity after 10 minutes of cooking at 100 ^{0}C. Those not soaked exhibited activity even after 45 minutes of cooking (Pusztai et al., 1987). Soaking for 24 hours followed by cooking for 20 minutes is effective in destroying TIA (Babar et al., 1988)

2.6.4 Methionine supplementation

Grain legumes are generally low in sulphur containing amino acids making methionine the first limiting amino acid in diets containing legumes. A large percentage of the sulphur containing amino acids in grain legumes are present as constituents of protease inhibitors and since the protease inhibiters are indigestible; then the availability of these amino acids in legume based diets is low. The situation is further exacerbated by increased excretion of proteases as part of protein inhibitor-enzyme complex. Proteases are rich in sulpur containing amino acids. The combined effects of the above actions would be a decreased performance due to a deficiency of sulpur containing amino acids (Dickson and Hosking, 1992). Methionine or cystine supplementation may therefore counteract much of the effects of the protease inhibitors. However since other ANF's such as lectins, tannins and NSP also contribute to depressed performance, supplementation may not totally reverse the effects (Wiryawan, 1997).

2.6.5: Enzyme addition

Today enzyme supplementation is routinely used in cereal-based diets to destroy the NSP in cereals (Charlton, 1996). Exogenous enzymes in use today are carbohydrases, proteases and phytases which are derived from fungi and bacteria. These enzymes are produced either through traditional submerged liquid fermentation (Bailey and Ollis, 1986) or solid state fermentation (Mitchell and Lonsane, 1992). Enzymes may improve the nutritive value of feeds in various ways: (a) through substituting the enzymes bound and lost by actions of ANF such as trypsin inhibitors hence maintaining and improving digestion, (b) through reducing the levels of microbes in the GIT by reducing substrate availability hence starving the microbes. Substrate reduction is achieved through breakdown of chemical bonds in feeds that are not usually broken down by the animal's own digestive enzymes hence releasing more nutrients. Enzymes also increase the availability of starches, proteins and minerals that are encapsulated by the indigestible cell wall fibre. In this respect the effects are similar to those brought about by antibiotic growth promoters, (c) through breakdown of antinutritive factors (ANF) that occur in feed ingredients.

Use of enzymes to destroy the ANF without affecting the dietary nutrients of legumes is a recent development. The inactivation of trypsin inhibitors and lectins of raw soybeans by incubation with microbial proteases in vitro has been reported (Huo et al., 1993). Ruminant fermentation processes have also been shown to destroy lectins and protease inhibitors (James et al., 1975). Woldetsadick et al. (1991) has demonstrated that the disappeared lectin activity in the rumen is not detectable in the small intestines even when the legumes constitute 75% of the dietary DM intake. These results suggest that the destruction of lectins is completed within the rumen. Hydrolysis and digestion of protease inhibitors is also completed within 24 hrs (Dixon & Hosking, 1992). Using a mixture of microbial enzymes (protease, cellulase, polygalactouronase and α-amylase) on raw field peas (V*icia faba L.),* Castannon and Marquardt (1989) reported similar broiler performance with enzyme treated, autoclaved or fermented field peas. Enzyme supplementation can also be used to digest the NSP in legumes to increase the metabolizable energy and protein values of these legume cereals (Pluske, 1998). Various trials done with proteases, carbohydrases or a cocktail mixture of the two have shown positive results (Castanon and Marquardt, 1989; Klein-Hessling, 2002). Using a cocktail of carbohydrases and proteases shows better results than each used separately due to the diversity of the NSP found in the cell walls (Walsh et al., 1997). The carbohydrate hydrolyzing enzymes disperse the protein NSP matrix leading to improved digestion of both. The NSP targeting enzymes may act through: i) partial hydrolysis of NSP as evidenced by reduced recovery of NSP when feedstuffs were incubated with enzymes in invitro studies (Meng et al., 2005), decreased digesta viscosity and rupturing of cell walls leading to increased accessibility to enzymes of encapsulated starch and proteins (Castanon et al., 1997)

Response to enzyme supplementation varies with the microbe used to produce the enzyme (Sunna and Antranikian, 1997); the enzyme mixture used (Pluske, 1998; Hruby and Pierson, 2002); source, species and cultivar of the feed ingredient (Bedford and Schulze, 1998). The ideal ratio of the enzymes needed to improve the nutritional value of grain legumes is specific for each legume (Wiryawan, 1997). The same enzyme produced by different microbes will require different conditions for optimum function while cell walls are also highly diverse in their structure.

Enzyme supplementation is likely to be a safer, more effective and cheaper method of reducing the negative effects of the antinutritive factors in grain legumes. The best enzymes to use and the extent of their effectiveness in improving animal performance however need to be elucidated (Wiryawan, 1997). As new and better enzymes are developed, it is essential to test their efficacy and safety rapidly to avoid abuse, speed up progress and also provide an effective alternative to antibiotics (Bedford, 2001), which are increasingly facing opposition from consumers (Pluske, 1998). The use of enzymes in feeds is only in its infancy and many exciting developments can be expected, particularly with the use of recombinant enzymes for a wide range of animals and animal feedstuffs. Enzymes not only will enable livestock and poultry producers to economically use new feedstuffs, but will also prove to be environmentally friendly, as they reduce the pollution associated with animal production (Marquardt and Han, 1997).

Allzyme SSF

This is a natural enzyme complex marketed by Alltech International through solid state fermentation. The microorganism used in the fermentation process is a fungus known as Aspergillus niger. Enzymes present in Allzyme SSF are; Amylase, Cellulase, Phytase, Xylanase, Beta-Glucanase, Pectinase and Proteases. The manufacturers recommend addition of this enzyme to poultry diets at the rate of 0.02% (Alltech, 2008).

2.6.6 Probiotics

Probiotics are a live microbial feed supplement fed to animals to promote productivity through improved intestinal microbial balance (Fuller, 1992). Probiotics are mostly selected and concentrated lactic acid bacteria and yeast (Stein and Kil, 2006). Like to growth promoters such as antibiotics, probiotics maintain a favourable micro flora in the alimentary tract by two strategies (a) competitive exclusion - attachment of probiotic microorganisms on the intestinal epithelial surfaces prevents pathogens from attaching (Stein and Kil, 2006) and (b) antagonistic activities towards pathogens. The effects are however brought about differently. Growth promoters tend to kill the organisms whereas the probiotics try to increase beneficial populations while inhibiting growth of the pathogens through production of bactericidal substances. Production of bactericidal substances such as bacteriocins, organic acids and hydrogen peroxides has been demonstrated (Jin et al., 1997). Probiotics may also prevent pathogens from utilizing nutrients in addition to increasing intestinal immunity against disease (Roselli et al., 2005). Safalaoh and Smith (1999) have reported increased body weight gain and feed conversion efficiency in broilers fed either probiotics or zinc bacitran (antibiotic), together or separately, implying that effective microorganisms is a potential replacement for antibiotic as a growth promoter.

Probiotics have also been reported to increase feed intake and digestion. Nahason et al. (1996b) found that supplementation of lactobacillus cultures in corn/soybean or corn/barley/soybean diets stimulated appetite and increased fat, nitrogen, calcium, phosphorous, copper and manganese retention in layers. Probiotics also improved gut health and absorptive area (Dunham et al., 1993) in addition to increasing bird resistance to heat stress (Zulkifli et al., 2000).

Impact of presence of microorganisms on digestive activity has also been demonstrated. Philips and Fuller (1983) have reported higher protease activity in intestinal digesta of conventional chicks as compared to germ free chicks. Twelve strains of *Lactobacillus* species isolated in chicken intestines were found to secret protease, amylase and lipase both intracellularly and extracellulaly (Jin et al., 1996b). The additive effect of microbial enzymes might be one of the reasons for the reported increase in feed intake and digestion observed with chicken fed probiotics. Probiotics are being introduced as alternatives to antibiotics but their effects in poultry have not been consistent giving rise to uncertainties (Jin et al., 1997). Microbial profiles in animals differ by species and age (Gaskins, 2001), therefore it is likely that different animal species and ages may require specific distinct probiotics tailored for them. Feeding pigs a probiotic made from lactobacilli isolated from weaned pigs reduced anaerobe counts and decreased diarrhea in weaning pigs (Huang et al., 2004) while using probiotic made from lactobacilli normally found in humans led to reduced growth and increased fecal excretion of pathogenic microbes (Lalles et al., 2007).

2.6.6.2 Effective microorganisms (EM)

EM is a combination of various beneficial, naturally occurring microorganisms mostly used for or found in foods. It contains beneficial organisms from 3 main genera: phototrophic bacteria, lactic acid bacteria and yeast; these effective microorganisms secrete beneficial substances such as vitamins, organic acids, chelated minerals and antioxidants when in contact with organic matter. At first, EM was considered an alternative for agricultural chemicals, but its use has now spread to applications in feed, environmental, industrial and health fields. Some of the benefits claimed to accrue from the use of EM include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on bird growth (Phillips and Phillips, 1996; Inciong, 1994). Increased egg production and egg weight and improvements in gross margins by up to 28.5 % have also been reported (Wei-jong et al., 1994**).** EM is registered and allowed for animal consumption in Austria, Sweden, Denmark, and China but it has not been registered in the USA. However, EM is consumed by humans in Japan and the USA as an antioxidant and probiotic health drinks.

Use of EM in Africa and Kenya in particular is new and in broiler production it is not backed by literature regarding use of microbial preparations. It is recommended that EM extended (mixture of 3% EM, 5% molasses end 92% water) be added to the drinking water for poultry at a ratio of 1:1,000 (http://www.mapleorgtech.com/what_is_em.html#poultry). However, Safalaoh and Smith (2006) reported that a higher dosage than this may be required to produce significant results. Their results suggest that a right dosage of 30 g EM/kg of feed is required to exhibit growth stimulation effects in broilers.

2.6.7 Prebiotics

While probiotics introduce good bacteria into the gut, prebiotics act as a fertilizer for the good bacteria that is already there. They help the good bacteria grow, improving the good-to-bad bacteria ratio.In other words, prebiotics are meant to provide a substrate for beneficial gastrointestinal microbes.Largeamounts of bacteriaare present in the monogastric small intestines and are potentially capable of utilizing theseindigestible carbohydrate sources for energy (Hajati and Rezaei, 2010). Prebiotics are defined as a non-digestible foodingredients that beneficially affects the host by selectivelystimulating the growth and/or activity of one or a limitednumber of bacteria in the colon (Gibson and Roberfroid, 1995). Since the 1980’s the possible potential effects of prebioticsin animal feeds was already recognized. Since then theinterest in the use of prebiotics in animal feed and petfood has resulted in a high research activity. The use ofprebiotics in diets for farm animals has been documented (Houdijk, 1998; Iji and Tivey, 1999 and Flickinger and Fahey, 2002). To date, all known and suspected prebiotics are carbohydrate compounds, primarily oligosaccharides, known to resist digestion in the human small intestine and reach the colon where they are fermented by the gut microflora. Studies have provided evidence that inulin and oligofructose (OF), lactulose, and resistant starch (RS) meet all aspects of the definition, including the stimulation of *Bifidobacterium*, a beneficial bacterial genus.Although all prebiotics are fiber, not all fiber is prebiotic. Classification of a food ingredient as a prebiotic requires scientific demonstration that the ingredient (Gibson and Roberfroid, 1995):

- Resists gastric acidity, hydrolysis by mammalian enzymes, and absorption in the upper gastrointestinal tract;

- Is fermented by the intestinal microflora;
- Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being.

An important mechanism of action for dietary fiber and prebiotics is fermentation in the colon and changes in gut microflora. The colonic environment is favorable for bacterial growth due to its slow transit time, readily available nutrients, and favorable pH (Cummings and Macfarlane, 1991). Together with the gut immune system, colonic and mucosal microflora contributes significantly to the barrier that prevents pathogenic bacteria from invading the gastrointestinal (GI) tract. Inulin, oligofructose, and FOS have been extensively studied as prebiotics, and have been shown to significantly increase fecal bifidobacteria at fairly low levels of consumption

2.6.8 Fermentation

In alcohol production, fermentation refers to the action of the enzyme zymase present in brewer's yeast to convert glucose sugar into ethanol and carbon dioxide. But in broad terms, fermentation refers to a whole series of chemical reactions brought about by microbes (bacteria, fungi and yeasts) to yield acids such acetic and lactic acids. In the process of rumen fermentation, the microbes break both starch and NSP into VFA's and lactic acids which can be used as sources of energy by livestock.

Fermentation is used in some parts of Africa to improve the nutritional value of beans by removing toxins. Inexpensive fermentation improves the nutritional impact of flour from dry beans and improves digestibility. Natural lactic acid fermentation has proved to be an effective method to decrease flatulence-producing compounds in beans. However, in order to use this method as a process on a large scale, it is fundamental to identify the microbial flora involved (Granito and Simon, 2006). When fermented seeds of *Phaseolus vulgaris* (black bean) were analyzed microbiologically, it was found that the microorganisms present were *Lactobacillus casei* and *Lactobacillus plantarum.* On performing induced fermentation with different inocula, a 63.35% decrease was found for the soluble fibre and 88.6% for raffinose, one of the main flatulence-producing compounds. When cooking under atmospheric pressure was applied to the fermented samples, a significant diminution of the trypsin inhibitors and tannins was found as well as an increase in the *in vitro* and *in vivo* digestibility of the beans.

These results demonstrate that the lactic acid bacteria used for the induced fermentation can lead to a functional food with improved nutritional quality (Granito and Alvarez, 2006). Partial degradation of nutrients in fermented feeds also make the feed easier to digest (pig international, 2004). Fermentation therefore directly improves feed digestion, nutrient availability and animal performance through destruction of ANF's and increased availability of bound nutrients.

Fermentation also produces acids which inhibit growth of harmful microbes both in the feed and in the gastro intestinal tract. A low *pH* has been shown to reduce the numbers of E. co7li and salmonella in the pigs' digestive tract (Pig International, 2004). With mounting pressure against use of antibiotic growth promoters, fermentation as a strategy of improving feed digestion and gut health may yet gain prominence. To achieve successful fermentation within a short time it is necessary to use an inoculant.

Bokashi

Bokashi is a composted material produced by culturing of bran or other substances, including agricultural or other wastes with extended effective microorganisms (EM). Extended EM for agricultural purposes is prepared from 3% EM1 and 5% sugarcane molasses in 92% clean water in airtight container(s) and left for fermentation for one to two weeks. A sweet-sour smell with pH of 3.5 or less indicates that EM extended solution is ready for use. Extended EM solution should be used within one month after its preparation (http://www.mapleorgtech.com/what_is_em.html#poultry). This mixture is then added to organic matter, like rice bran, and mixed thoroughly until it becomes approximately 30% moist. It is recommended that it should be moist enough to form a clump which sticks together if you squeeze some in your hand, but should be dry enough to crumble again when touched lightly. It is then left to ferment for one to two weeks depending on temperature. A pleasant sweet-sour smell indicates that the process is complete. Different types of EM Bokashi can be made1from different organic materials and used for different purposes. The most often used ingredients are rice/wheat bran, rice/wheat husks, oil cakes, fishmeal, etc. (http://www.mapleorgtech.com/what_is_em.html#poultry). EM has become very popular in the poultry and fish industry. Feed is fermented with EM before feeding. A variety of feeds from various feeds can be prepared using EM. To ferment the feed, the same process as that for making Bokashi is used.

2.7 Additives in animal feeding

Today the meat and food industry is facing increasing scrutiny from consumers worried about residues in meat, food poisoning, animal welfare and pollution (Lyons, 1998). Most of these problems have arisen from intensification of production methods and the need to reduce stress, improve production and profits. Antibiotics are the most common of these feed additives used at below therapeutic levels to maintain the balance of microorganisms in the GIT in favour of non-pathogens. Banning use of antibiotics without a substitute is likely to erode the gains made towards improving productivity of intensively kept animals (Klein-Hessling, 2002). However, there are natural alternatives such as enzymes and probiotics, which might take up the place of antibiotics (Bedford, 2001). Enzymes destroy the ANFs, improve digestion and also lower levels of harmful microorganisms (Wiryawan, 1997; Bedford, 2001). Bedford (2001) actually reports that most of the enzymatic performance response observed could be due to changes in the intestinal micro flora rather than due to a direct effect of enzymes *per se* on diet digestibility. Probiotics on the other hand enhance the population of useful micro-organisms; improve absorption and also secret bacterial enzymes into the GIT (Jin et al., 1996a). It is therefore possible that the probiotics can be used interchangeably with enzymes to replace antibiotics. Indeed using probiotics would be equivalent to indirectly adding bacterial enzymes to complement host's enzymes. It is therefore possible that probiotics might actually destroy the ANF'S in feeds as evidenced by ANF destruction in the rumen of ruminants.

CHAPTER THREE

3. NUTRITIONAL CHARACTERIZATION OF COMMON BEAN (*Phaseolus vulgaris*) VARIETIES GROWN IN KENYA AND THE EFFECT OF PROCESSING ON THEIR NUTRITIONAL QUALITY

3.1 Abstract

This experiment was designed to investigate the nutrient composition and anti-nutritive factors of five most popular common bean varieties (*Phaseolus vulgaris*) grown in Kenya. In addition the experiment also investigated the effects of cooking, soaking overnight followed by cooking and solid state fermentation on nutritional and anti-nutritive composition of one of the bean varieties. Samples of bean varieties were randomly purchased from vendors in Nakuru municipal market. Samples within each cultivar were pooled together and thoroughly mixed. The seed samples were cleaned of any extraneous materials, and subsequently ground to pass through a 1-mm screen. Representative samples were taken for laboratory analysis. The samples were analyzed for proximate composition, energy, starch and ANF's. All analyses were done in triplicate. Red haricot bean variety was further subjected to various treatments and analyzed for both nutrititive and antinutritive composition. A sample of red haricot bean variety was either cooked directly or soaked overnight before cooking in boiling water. The samples were analyzed for lectin activity and TIA at ten minute intervals up to forty minutes. Another sample of red haricot beans was ground into floor and fermented with effective microorganisms (EM) for fourteen days and then analyzed for lectins, TIA and proximate composition. The results indicated that common beans grown in Kenya are high in proteins (18-23%) and energy (2500-2700kcal). The beans are also rich in lectins (4 to 16 HU/mg) and TIA (2.13 to 3.04 TIU/mg). Crude fibre ranges from 6 to 8%. The results also indicated that soaking red haricot beans for 24 hours followed by cooking in boiling water for 30 minutes was effective in eliminating TIA activity and reducing lectins to safe levels. Fermentation reduced lectins by 94% and TIA by 22%.

Key words: anti-nutritive factors, common beans, cooking, fermentation, nutritional composition

3.2 Introduction

Common beans were probably introduced into Africa by the Portuguese and spread into the interior faster than European exploration. Common Beans (*Phaseolus vulgaris)* originated from South and Central America where its wild forms are found over a wide discontinuous range from Argentina to Mexico. There are morphological and biochemical differences between the populations in Mexico and those in South America and in fact hybrids between plants from the two regions are largely sterile. This sort of evidence is good enough to place them in different species but this has not been done because they both gave rise either separately or in combination to the various cultivars of beans now placed in *Phaseolus vulgaris* (Wortmann et al., 1998). Analysis of seed proteins, isozymes and differences in morphology of *P. vulgaris* cultivars show that this species has six major races that are the result of six separate domestication events between Mexico and the southern Andes. There has been some subsequent hybridization between these races to produce further varieties (Wortmann et al., 1998).

The diversity of common bean seed types in Africa has been reported as massive but varies across the region (Njuguna et al., 1980; Wortmann et al., 1998). It is highest (more than 10 varieties) in pure subsistence systems practiced in the great lakes region (Rwanda, Burundi and Democratic Republic of Congo) and Southern Uganda and reduces with a higher degree of bean production commercialization in the Central Rift Valley of Ethiopia. Wortmann et al. (1998) classified common bean varieties into 9 major classes according to colour and size as: pure large reds, medium and small reds and red mottled, purple, yellow and tans, cream, navy/white and black. Spatial distribution of seed types in Eastern and Southern Africa is a result of many factors but market forces and agro-ecological conditions are major contributing factors. The reds haricot and red mottled (Rosecoco) beans are the most common types due to market preferences. Wortmann et al. (1998) estimated an aggregate area share of about 50 percent for pure reds and red mottled in Eastern Africa and about 27 percent in southern Africa.

In the developing world and Kenya in particular, beans are a staple food of the poor (usually eaten with ugali, whole maize or rice). The young pods may also be used as green beans. Rice, maize or maize meal (Ugali) served with beans are often a staple meal where there is limited money for meat. The amino acids in this combination make it a complete protein source (Katungi, 2009).

Common beans can also be an excellent source of protein and energy for poultry and pigs. The nutrient composition of these beans from other parts of the world, especially Western Europe are well documented and widely accepted by stock feed manufacturers, especially in Europe where these legumes have long been used for animal feeding (Rubio et al., 2003). However, whilst such overseas data could be useful, it is inadequate for accurate feed formulations under Kenyan conditions. Interactions of cultivars, soil, climate and agronomic factors can cause appreciable differences in nutrient profiles between locally grown ingredients and those available elsewhere (Valdebouze et al., 1980; Cedvez and Gokgoz, 2007)

Another factor which limits the utilization of common beans in poultry diets is the uncertainty about their nutritional quality (Wiryawan, 1997). The variations reported in the nutritive value of grain legumes are partly due to variable amounts of anti-nutritive factors that interfere with nutrient digestion and bird performance. The anti-nutritive factors commonly found in grain legumes include protease inhibitors, lectins, tannins, amylase inhibitors and non-starch polysaccharides (Choct, 2006). As a result, feeding raw legumes generally results in poor growth and feed efficiency in poultry (Kakade et al., 1974; Olkowski et al., 2005). However, each legume produces a different response due to varying quantities of ANF's (Viveros et al., 2001). Most current legume cultivars have been bred for low levels of these anti-nutritive factors. Some of the common bean cultivars grown in Kenya are of unspecified origin and may belong to cultivars with significant levels of anti-nutritional factors. These toxic compounds are reported to be especially concentrated in red kidney beans. For this reason, locally grown cultivars need to be evaluated for the levels of both nutrients and anti-nutritive factors in both *in vitro* and *in vivo* trials.

Many attempts have been made to reduce the levels of these anti-nutritive factors in common beans. Soaking in water and salt solution is a common practice to soften texture and hasten the cooking process in addition to improving the nutritional quality of legumes (Silva et al., 1981). Beside improvement in nutritional quality some valuable nutrients may be lost during processing. Losses of minerals and vitamins have been found more significant as compared to protein and other nutrients (Silva et al., 1981). In this context, this study was designed to find out the effect of soaking and cooking on the nutritional quality of common beans.

The aims of this experiment were therefore:

I) To determine the nutrient and anti-nutritive composition of five most common bean cultivars grown in Kenya.

II) To assess the effects of boiling, boiling time and fermentation on the nutritional and anti-nutritive properties of common beans.

3.3 Materials and methods

3.3.1 Sample collection and preparation

A total of 30 bean samples representing five cultivars (six samples for each cultivar) of the most common bean varieties available at the Nakuru municipal market were collected for analysis. Samples of bean varieties were randomly purchased from vendors in the market. Samples within each cultivar were pooled together and thoroughly mixed. The common names of the collected samples were Rose coco, Mwezi moja, Red bean, Mwitemania and New mwezi moja. The seed samples were then cleaned of any extraneous materials, and subsequently ground to pass through a 1-mm screen. Representative samples were then taken for laboratory analysis. The samples were analyzed for proximate composition, energy, starch and ANF’s. All analyses were done in triplicate. A brief description of the beans collected is indicated below.

GLP 2 Rosecoco

Reddish bean with white/creamy spots. A high yielder ideal for medium altitudes (1500-2000) and high rainfall areas. The variety has wide adaptability. Takes 2 to 3 months to mature and has yield potential of 1.8 to 2 tons per hectare.

.Rosecoco

GLP 1004 Old Mwezi Moja (Kifuu mrefu)

Well suited for the drier semi-arid low rainfall areas and also performs well in medium rainfall areas during short rains. Gives good yields even with little rain. Seeds are large beige or light brown speckled purple. Tolerant to drought. Altitude Range 1200-1600. Duration to Maturity is 2 to 3 months. Grain Yield is between 1.2 to 1.5 tonns/ha.

Mwezi moja

GLP 585: Red haricot (Wairimu)

This is commonly referred to as Wairimu. It is also sometimes referred to as small red bean or Mexican red bean. These are similar to red kidney beans (Canadian wonder, GLP24), only smaller, rounder, and darker. The beans are heat tolerant, good for maize intercropping and have excellent cooking qualities. Altitude Range is 1500-2000. Duration to maturity is 2.5 to 3 months. Grain yield is 1 to 1.5 tonns/ha.

Red haricot

GLP 92 Mwitemania

In Kenya the bean is very popular, seeds are broad, creamish coloured with dark brown spots, tolerant to halo blight and very high yielder. Good for medium altitude areas. Growth habit is bushy. It is popularly known as “surambaya”. Wide adaptability and resistant to hallo blight Altitude Range of 900-1600 m. Duration to maturity of 2 - 3 months. Grain yield is 1.2-1.5 tonns/ha.

Mwitemania

GLP X1127 New Mwezi Moja

Dark brown creamy, flecks. Altitude range 1000-1500. Duration to maturity 2-3. Grain yield 1-1.5 tonns per hectare.

New mwezi moja

3.3.2 Processing methods
Soaking

Red Haricot (Wairimu) beans were soaked in distilled water in a glass jar for 24 hours. Forty grams of bean sample were placed in a one litre capacity glass jar. Distilled water (800 ml) was added and the mixture stirred for 1 minute then left to stand for 24 hours.

Cooking time

Pre-soaked or unsoaked whole seeds were cooked in a beaker in boiling distilled water for varying time periods. Samples were allowed to cook in boiling water for 10, 20, 30 or 40 minutes. The ratio of sample to water was 1:20 (w/v).

Fermentation

Using EM technology of making bokashi as described in the literature review page 26, the beans were ground and ensiled in 1000 mm gauge polythene tubes for 14 days. On the 14^{th} day, the fermented flour was removed from the tubes and oven dried at 60^{0}C. The dried material was then ground to break the clumps. The flour was used for analysis.

3.3.3 Analytical methods

Composition

Dry matter, starch, sugar, energy, ash, ether extract, crude protein and crude fibre were determined using near infrared systems (NIR). The NIR system used was 5000 model with a segment range of 1100-2498 and calibrated with NIR instrument model 6500. NIR is a spectrometer which can analyze both liquid and solid samples. The system is based on Beer-Lambert law which states that the absorbed light is directly proportional to the concentration of the chemical in the sample. Uniformly ground samples were first heated at 60 ^{0}C to obtain uniform moisture content. The samples were then poured into cuvets or sample cells and compacted then analyzed.

Lectin activity

Lectin activity was measured as agglutination activity using rabbit erythrocytes as described by Valdebouze et al., (1980). Rabbit red blood cells were washed several times by centrifugation until a clear supernatant was obtained. Trypsin was then added (30 minutes at 37^{0} C) to the washed suspension that contained 50% red blood cells. The suspension was then rewashed and diluted to an absorbance 0.500 at 620 nm.

One gramme of finely ground sample was then vigorously shaken for five minutes with 50 ml of haemagglutinating buffer and allowed to stand overnight at 4 ^{0}C. Difco haemaglutinating buffer (Wheeler et al., 1950) with NaCl, Na_2HPO_4, and KH2PO4 was used. The suspension was then centrifuged and the supernatant subjected to successive serial dilutions (1:1) with the same buffer. An equal amount (1 ml) of trypsinized red blood cells suspension was then added to each dilution, which was then left overnight at 4^{0} C. To evaluate the resulting agglutination the agglutinated cells were estimated visually. Haemagglutinin activity was expressed as haemaggutinin units per miligramme of sample. One haemagglutinin unit is defined as the smallest amount of sample necessary for agglutination under the test conditions. Total activity was defined as the inverse of the highest dilution still capable of causing agglutination. Specific activity was expressed as hemagglutination units per mg of sample.

Trypsin inhibitor activity (TIA)

TIA was determined using the procedure of Kakade et al. (1974) as modified by Valdebouze et al. (1980). One gram of finely ground sample was suspended by constant stirring for about 30 minutes in 100 ml of water adjusted to a pH of 2.9 with hydrochloric acid. Portions of 0, 0.6, 1.0, and 1.4 ml of sample suspension was pipetted into graduated test tubes and adjusted to 2ml with water. To each tube, 2 ml of trypsin solution and 5 ml of α-N-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) solution (prewarmed at 37^{o}C) was added respectively to promote the formation of an enzyme-inhibitor complex. The tubes were then incubated in a water bathe at 37^{0}C. The reaction was terminated 10 minutes later by adding 1ml of 30% acetic acid, mixed and filtered. The absorbance of the filtrate was then measured at 410 nm against a reagent blank prepared by adding 1ml of 30% acetic acid to test tube containing 2 ml water and 2ml trypsin solution. Trypsin inhibitor activity (TIA) was expressed in units of trypsin inhibited (TUI) per milligram of sample.

3.3.4 Data analysis

Each determination was carried out in triplicate and figures were then averaged and data presented as means.

3.4 Results

3.4.1 Chemical composition of common beans grown in Kenya

Composition

In this experiment, the five most common varieties selected for analysis were: GLP2 (Rose coco), GLP1004 (mwezi moja), GLP 585 (Red Haricot or Wairimu), GLPX92 (Mwitemania) and GLP1127 (New mwezi moja). These bean varieties were subjected to analysis in order to determine their nutrient profile; mainly crude protein, energy, starch, total sugars, total lipids and fibre as shown in Table 3.I.

Table 3.1 composition of five bean varieties collected at Nakuru municipal market

Bean variety	**GLP2**	**GLP1004**	**GLP585**	**GLP92**	**GLP1127**
Common name	Rosecoco	Mwezi Moja	Red haricot	Mwitemania	New Mwezi Moja
Dry matter (%)	88.21	88.19	88.48	88.43	88.03
Crude protein (%)	22.95	20.03	23.48	18.21	20.82
Energy (Kcal/Kg)	2631.45	2573.90	2687.93	2595.01	2616.99
Crude fibre (%)	6.76	8.34	7.09	7.33	8.11
Crude fat (%)	1.32	1.55	1.28	1.49	1.33
Starch (%)	38.30	38.13	38.27	40.93	39.93
Sugar (%)	3.85	3.89	4.06	3.21	3.43
Lysine (ppm)	12.87	10.33	13.01	8.67	10.65
Tryptophan (%)	2.00	1.87	2.13	1.59	1.85
Methionine (%)	7.17	5.93	7.16	5.76	6.77

The data were not subjected to statistical analysis since the aim was to present an overview of relative nutritive values rather than to provide a statistical comparison. Protein and starch are the main components of these common beans. Notable variations could be observed in CP (varied from 18% to 26%), starch (varied from 35% to 41%) and lysine content (varied from 0.87% to 1.78%). Other chemical components did not vary much. Huma et al. (2008) have reported values of 23.69% CP, 4.7% lipids, 7% sugars, and 1.7% fibre for red kidney beans grown in Pakistan. These figures are higher for fat and low for fibre compared to values obtained here. In this experiment, fibre ranged from 6.8% to 8.3% while fat content ranged from 1.3 to 1.6%.

Lectin and trypsin inhibitor activity

The lectin and trypsin inhibitor activity in the beans analyzed is shown in table 3.2. The lectin activity varied from 4 HU/mg to 16 HU/mg. The red Haricot (Wairimu) had the highest agglutination activity (16 HU/mg) while GLP1004 (Mwezi moja) had the lowest activity (4 HU/mg). Trypsin inhibitor activity varied between seed varieties from a low of 2.13 TIU/mg in GLP1127 to a high of 3.04 TIU/mg in red haricot.

Table 3.2 Lectin and trypsin inhibitor activity of common bean varieties grown in Kenya

Bean Variety	Sample (mg)	Total activity (HU)*	Specific activity (HU. Mg$^{-1)**}$	Minimum agglutination capacity (mg)***	TIA(TIU/mg)
Rose coco	1000	8000	8.0	0.125	2.352
Mwezimoja	1000	4000	4.0	0.25	2.240
Red haricot	1000	16000	16.0	0.0625	3.04
Mwitemania	1000	8000	8.0	0.125	2.768
NewMwezi Moja	1000	8000	8.0	0.125	2.128

* Inverse of the highest dilution capable of causing agglutination.

** Hemagglutination units per mg of sample

*** Minimum agglutination capacity- minimum amount of sample that is able to agglutinate enzyme-treated rabbit erythrocytes

3.4.2 Effects of processing on nutritive and anti-nutritive content of red haricot variety

Red haricot variety was used for this test. This variety was chosen for its high protein content and ready availability. The processing methods applied were cooking and fermentation

Composition

The results of nutritive composition of processed red haricot beans are presented in table 3.3 below. In this experiment, boiling beans without soaking reduced fibre, proteins and crude fat by 8.4%, 11.7% and 20% respectively. This treatment also reduced sugar by 2.3% while starch increased by 7.3%. Boiling beans for 30 minutes after soaking for 24 hours also influenced the nutritive content of the beans. Soaking and boiling reduced fibre, proteins and crude fat by 4.5%, 10.6% and 53% respectively. This treatment also reduced sugar by 4.7% while starch increased by 8.5%. Fermentation for 14 days reduced crude fibre, protein and fat values by 18%, 12.5%, and 53% respectively. Fermentation also increased starch and sugar values by 6.2% and 6.9% respectively.

Table 3.3 Effects of processing on nutrient composition of red haricot beans

Item	Soaked Boiled	Boiled	Fermented	Raw
Dry Matter (%)	88.45	88.50	89.45.	89.85
Crude Protein (%)	23.48	23.31	23.13	26.36
Energy (kcal)	2687.93	2721.43	2715.53	2701.82
Crude fiber (%)	7.09	7.62	6.80	8.29
Crude fat (%)	1.28	1.24	0.70	1.50
Starch (%)	38.27	38.03	37.58	35.39
Sugar (%)	4.06	4.22	4.61	4.30
Lysine (ppm)	13.01	12.31	13.15	17.75
Tryptophan (ppm)	2.13	2.02	2.30	2.53
Methionine (ppm)	7.16	7.23	7.48	7.87

Lectin and TIA activity

The results indicate that fermentation had the greatest effect on lectin activity. Fermentation reduced lectin activity by 94% while soaking and heating reduced lectin activity by 75%. Soaked and boiled beans recorded a maximum reduction in lectin activity of 75% at 20 minutes of boiling. Further heating had no effect on lectin activity. Unsoaked boiled beans recorded a maximum reduction of activity (50%) at 10 minutes. Extra heating time had no effect on lectin activity.

The results also indicate a complete elimination of TIA on soaking beans for 24 hours followed by 30 minutes of cooking in boiling water. Boiling unsoaked beans however could not completely remove TIA. Even 40 minutes of cooking only reduced TIA by 93%. On the other hand fermentation had little effect on the TIA activity. After fourteen days of solid state fermentation of red haricot beans, the TIA activity reduced by 22%.

Table: 3.4 Effect of processing red haricot beans on lectin and TIA activity

Item	Boiling Time (minutes)	Sample (mg)	Total[a] activity (HU)	Specific[b] activity (HU/mg)	Minimum[c] agglutination capacity (mg)	TIA (TIU/mg)
Raw	0	1000	16000	16.0	0.0625	3.040
Soaked &boiled	10	1000	8000	8.0	0.125	1.031
‘’	20	1000	4000	4.0	0.250	0.410
‘’	30	1000	4000	4.0	0.250	0
‘’	40	1000	4000	4-0	0.250	0
Dry& boiled	10	1000	8000	8.0	0.125	1.031
‘’	20	1000	8000	8.0	0.125	0.410
‘’	30	1000	8000	8.0	0.125	0.410
‘’	40	1000	8000	8.0	0.125	0.205
Fermented	14 days	1000	1000	1.0	1.0	2.384

[a] Inverse of the highest dilution capable of causing agglutination.

[b] Hemagglutination units per mg of sample

[c] Minimum agglutination capacity; minimum amount of sample that is able to agglutinate enzyme-treated rabbit erythrocytes

3.5 Discussion

3.5.1 Composition

A high diversity of common bean seed varieties has been reported to exist in Kenya. About 80 different seed varieties have been distinguished in different regions of the country (Njuguna et al., 1980), but six are most popular. They include: i) Red and red/purple mottled types which are known in different local names such as Roseccoco, Nyayo, Wairimu, Kitui etc, ii) Purple/grey speckled locally known as Mwezimwoja and iii) Pinto sugars locally known as Mwitemania or Surambaya. According to Njuguna et al. (1980), Rosecoco was the most widely grown followed by Canadian wonder types at the time. Rosecoco and Canadian wonder types are high yielding but require heavy rains and high soil fertility to yield well. Consequently, these varieties have been losing area because of increased problem of soil fertility and associated diseases and are being replaced by varieties like large Pinto "sugar bean" locally called "Surambaya" and red haricots that are well adapted to poor soil conditions. In this experiment, the five most common varieties selected agree with the report of NJuguna et al. (1980), who classified them as amongst the most popular. The investigated varieties were: GLP2 (Rose coco), GLP1004 (mwezi moja), GLP 585 (Red Haricot, Wairimu), GLPX92 (Mwitemania) and GLP1127 (New mwezi moja).

The beans investigated here had variable composition. This is in agreement with values for beans reported by other researchers. Wang et al. (2009) have reported a red haricot bean composition of 24.1% protein, 38.8% starch, 3.9% ash, 1.4% fat, 21.6% fibre and a borlotti (rose coco) bean composition of 24.0% protein, 39.5% starch, 4.1% ash, 1.3.% fat, 14.6% fibre. Other authors have reported a pinto bean composition of 18.8-22.4% protein, 1.0-1.2% fat, 61.8% carbohydrates (42.5% starch), 6.3-18.9% fibre, 3.5-3.8% ash (Meiners et al., 1976; Wang et al., 2009). Legumes generally contain low fat contents in the range of 1 - 2% with the exception of chickpea- 6.7%, soybean- 21% and peanut- 49% (Augustin and Klein, 1989). Lipid content in legumes mainly depends upon variety, origin, location of growth, climate, season, environmental factors and soil type.

After processing, chemical composition of the beans changed. After fermentation, crude fibre, protein and fat values reduced by18%, 12.5%, and 53% respectively. These results agree with Granito and Alvarez (2006) who reported that fermentation of black common beans (*Phaseolus vulgaris*) variety L-140 resulted in a reduction of crude fibre (40%), fat (37%) and proteins (4.51%) as observed here. Some authors have demonstrated that the decrease in soluble and insoluble fibre during fermentation processes in legumes could be attributed to the hydrolysis of pectic compounds and the use of cellulose and hemicellulose as substrates by the microorganisms responsible for the fermentation (Granito et al., 2002). Likewise, it has been reported that the hydrolysis produced on the fibre during fermentation can trigger an increment in the concentrations of glucose and maltose, which are later transformed into organic acids or alcohols through the microbial activity (Idris et al., 2001; Siebenhandl et al., 2001). In this study sugars increased by 7%. These results also agree with Granito and Alvarez (2006) who also reported that fermentation increased sugars and overall diet digestibility. Decrease in protein, starch and fats could also be due to hydrolysis by the microbial enzymes.

In this experiment, cooking beans with or without soaking reduced proteins and crude fibre. Emiola et al. (2007a) reported that cooking reduced the CP content of common beans possibly due to leaching and vaporization of some nitrogenous compound during cooking. Cooking is also effective in reducing lectins and TIA to safe levels.

3.5.2 Lectins

All the beans analyzed contained lectins. The lectin activity varied with bean variety. These results may be attributed to genetic differences in common bean varieties, climate, environment, geographical factors, which could affect the content and activity of the common beans. The occurrence in nature of erythrocyte-agglutinating proteins has been known since the turn of the 19th century. These cell-agglutinating and sugar-specific proteins have been named lectins and shown to occur widely in plants and to some extent also in invertebrates. To date numerous lectins have been isolated from plants as well as from microorganisms and animals, and during the past two decades the structures of hundreds of them have been established. The hemagglutination induced by lectins is a consequence of a reaction of the plant protein with carbohydrates on the surface of the red cells.

Among the numerous lectins studied so far, those from legumes represent the largest family. They can be present at relatively high amounts depending on genetic as well as environmental factors, and are accumulated especially in the seeds (Nasia et. al., 2009). Legume lectins show an extensive homology in their primary structures, although they possess different carbohydrate specificities based on the amino acid sequence of the active site (Young & Oomen, 1992; Sharon, 1993). In the seeds of the common bean *P. vulgaris*, the protein fraction which has sugar binding property and hemagglutinating ability (Goldstein et al., 1978) is called phytohemagglutinin (PHA). *Phaseolus vulgaris* (kidney bean) contain 1–10g/kg lectin. The concentration varies with both genetic and environmental factors (Peumans and Van Damme, 1996). PHA binds to the intestinal mucosa of rats, resulting in the appearance of lesions, disruptions, and abnormal development of the microvilli (Liener, 1986). When PHA is added to the diet of experimental animals, it inhibits the absorption of nutrients across the intestinal wall and greatly increases the bacterial colonization of the small intestine. Significant variations in the content of lectins have been observed in plants, due to environmental influences, such as drought or salinity stress (De Souza Filho, 2003). The normal range of lectin levels in *P. vulgaris* is 1–10 g/kg (and up to 5% seed dry weight) (Peumans and Van Damme, 1996). Some bean cultivars have not have a detectable level of lectins in seeds, a trait inherited as a recessive allele (Peumans and Van Damme, 1996).

The most common method of lectin destruction is in boiling water at atmospheric pressure or in autoclave (Alonso et al., 1998). The hemoagglutinating activity of bean lectins is progressively reduced with heat treatment, and thermal degradation is better if bean seeds are pre-soaked before boiling (Grant, 1982). This is in agreement with the current results where soaking resulted in a marked decrease in hemagglutinin activity as compared to direct heating. In this experiment boiling of soaked beans under atmospheric pressure produced a reduction in lectin activity of 75% within 20 minutes while boiling dry (unsoaked) beans for the same period produced a 50% reduction in lectin activity. No further reduction was observed even when heating continued for 40 minutes. Bean PHA has been reported to resist even drastic microwave treatment (Hernandez-Infante et al, 1998). On the other hand Stelio et al., (2002), reported that when lectins of red marine alga (*P. capillace*) in solution was heated at 80^{o}C for 10 min, the hemagglutinating capacity of the lectin declined rapidly, reaching 6% of the control value. The hemagglutinating activity was totally destroyed by heating the lectin at same temperature for (80^{0}C) 30 min. Ma and Wang (2010) however reported that even after heating at 100^{0}C for 20 minutes soybean lectins still retained 47%

activity. Puztai and Grant (1998) also reported that despite the heat treatment of soybeans, findings have shown that it still contains low-levels of anti-nutritional factors, such as phytoagglutinin. This implies denaturation alone is not effective to fully deactivate SBA (soybean agglutinin). In addition, there was no significant difference between boiling at 100^0C for 20 minutes or autoclaving at 121^0C for 30 minutes to denature SBA. These results agree with the current findings where further boiling beyond 10 minutes for unsoaked or 20 minutes for soaked beans had no further reduction in lectin content. Fermentation produced the highest decrease in lectin activity of 94%.

3.5.3 Trypsin inhibitor activity (TIA)

The results indicate a complete elimination of TIA on soaking for 24 hours followed by 30 minutes of cooking in boiling water. Emiola et al. (2007) also reported that boiling red kidney beans in water reduced TIA better than toasting. Boiling unsoaked beans even for 40 minutes reduced TIA by 80%. Vidal-Valverde et al. (1997) reported that cooking presoaked faba bean seeds for 35 min in distilled water resulted in a 100% TIA loss while direct heating resulted in less reduction of TIA by 69%. Nestares et al. (1993) have also reported that cooking of chick peas soaked in either water, acidic, or alkaline solutions resulted in complete elimination of TIA, while direct heating caused only a 27% decrease in TIA. Most compounds with protease inhibitor activity respond to heat treatment, but the thermo stability of TI in legumes has been reported to vary not only with legume source but also with the different conditions used during processing, such as pH, humidity, time, temperature, and pressure (Diaz-Pollan et al., 1997).

Results of fermentation show a partial elimination of TIA. The TIA activity reduced by 22% after fourteen (14) days of solid state fermentation. This is in contrast with Stodolak and ska-Janiszewska (2008) who reported that solid state fermentation of grass-pea seeds reduced TIA by 99%. However, the grass seeds were first boiled in tap water for 30 minutes before fermentation. Ibrahim et al. (2002) also reported that solid state fermentation completely removed trypsin inhibitor, oligosaccharides and reduced remarkably phytic acid from cowpeas precooked in boiling water for 20 minutes. Ibrahim et al. (2002) also reported that cooking alone reduced the level of trypsin inhibitor in cowpeas by 86.98 to 90.46%. A combination of boiling in water and fermentation may have contributed to the complete elimination of TI reported by the Authors. Solid state fermentation without precooking may not allow adequate contact between the microbial enzymes and the substrate for effective hydrolysis to occur (Thorpe and Beal, 2001).

Liquid state fermentation has proved very effective in TIA elimination (Hou et al., 1993). Huo et al. (1993) have demonstrated that fungal and bacterial protease enzymes in solution could inactivate trypsin inhibitors and lectins in raw soybean (RSB) and low temperature extruded soybean (LTES) *in vitro*, to various extents. The most effective of these enzymes reduced trypsin inhibitor levels in RSB and LTES by 96% after 12 hours incubation at an inclusion level of 1%. The bacterial proteases appeared to be more effective at breaking down trypsin inhibitors than the fungal protease. Meijer and Spekking (1993) isolated microorganisms producing enzymes that could utilize purified sources of Kunitz soybean trypsin inhibitor (KSTI) and Bowman-Birk inhibitor (BBI) as sole carbon, nitrogen and sulphur sources, and inactivate them over a time period of several days. Therefore these microorganisms must all possess enzymes capable of hydrolyzing the trypsin inhibitors in soybeans.

It is therefore possible that the low level of TIA elimination observed in this experiment after fermentation could be partly due to low contact between the substrate and bacterial enzymes due to solid state fermentation. Liquid state fermentation does not appear to have attracted so much attention because liquid feeding systems are generally not used in poultry (Thorpe and Beal, 2001).

3.6 Conclusion

An extensive range of feed materials are used in formulating rations for livestock and some of them are, to a greater or lesser extent, interchangeable with maize, maize by-products or soybean meal. The main factors that determine the scope for substitution are the nutrient composition of the feed (e.g. energy concentration, the levels and digestibility of amino acids, mineral content etc), and any deleterious aspects e.g. the presence of anti-nutritive compounds or low palatability of the feed. These factors combined determine the fitness and scope for one feed to replace another. Any feed material used to replace maize, maize by-products or soybean meal must therefore provide the nutrients they are replacing.

FAO (2008) reported that Kenya is the largest producer of common beans in Africa (500,000 metric tons) followed by Uganda and Tanzania. All the beans produced in Kenya currently are mainly for human consumption (100%). Between 2001-2009, production of common beans in Kenya grew at a rate of 5.2 percent with the area expansion (at an average rate of 3.3 percent per year) as the main source of this growth. The area is forecast to continue to increase although with some moderation in the rate of increase to below the current rate of 3.3 % in the next 10 years due to land shortage associated with population pressure (Katungi, 2009). This therefore suggests that Kenya will continue to produce large quantities of common beans for the foreseeable future

From the results of this study common beans grown in Kenya, are a good source of protein (18-26%) with good levels of lysine but low in methionine and cysteine relative to SBM. Soya bean meal (SBM) is probably the best quality vegetable protein source used as a livestock feed in the world. SBM has high energy concentrations as a result of the high digestibility of the fibre and protein concentrations, and good levels of essential amino acids, particularly lysine. Common beans, compared to SBM, have a lower protein content which can also be variable depending on the variety, but on an equivalent total protein basis the two feeds are comparable in terms of essential amino acids. Common beans are high in energy (from starch) and low in fibre content which makes it possible to use them in poultry rations.

Due to the presence of antinutritive factors in the common beans it means that some form of processing is necessary before they can be utilized. Processing through heat treatment and fermentation can be used to reduce the content of the anti-nutritive factors in common beans. Fermentation marginally reduced the content of trypsin inhibitor in red haricot beans but caused substantial reduction in Haemagglutinin. On the other hand heating beans in boiling water inactivated trypsin inhibitor and haemagglutinins. This is in agreement with results of Emiola et al. (2007). Cooking and fermentation however tended to reduce the CP content possibly due to leaching and vaporization of some nitrogenous compounds during processing.

From the results of this study, it is apparent solid state fermentation has a limited effect on protease inhibitors but quite a drastic effect on lectins. Many other benefits are also attributed to fermentation such as reduction in fibre content. It also preserves and enriches food, improves digestibility, and enhances the taste and flavour of foods. It is also an affordable technology and is thus accessible to all populations. Furthermore, fermentation has the potential of enhancing food safety by controlling the growth and multiplication of a number of pathogens in foods. These benefits make fermentation an attractive system of processing common beans.

Cooking treatments are also effective to varying extents in reducing the effects of ANFs. Boiling alone is not enough to remove the lectin activity completely. Pre-soaking before boiling results in lower lectin levels than direct boiling. Cooking beans in boiling water for 30 minutes however reduces TIA to undetectable levels.

Based on the results reported here, the following conclusions can be made:

1. Locally grown common beans have levels of ANFs comparable to reported figures

2. The quality and quantity of common beans produced locally is sufficient to offer a viable substitute to SBM in animal feeds provided ANFs are neutralized.

4. Processing of red haricot beans through fermentation is partially effective in reducing ANFs

3. Soaking red haricot beans for 24 hours followed by cooking in boiling water for 30 minutes completely eliminates TIA and reduces lectins to safe levels.

CHAPTER FOUR

4. EFFECTS OF ENZYME ADDITION ON ANTI-NUTRITIVE FACTORS AND UTILIZATION OF RAW OR PROCESSED RED HARICOT BEANS BY BROILERS

4.1 Abstract

The effects of oven heating soaked red haricot beans, fermentation and multienzyme supplementation on broiler performance were investigated in a 28 day trial. Enzyme was added to the diets at the rate of 0.02%. Two hundred and eighty (280) seven day old broiler chicks were distributed among seven diets in a completely randomized design to give four replicates per diet (10 chicks per replicate). The diets contained maize, fish meal and either SBM (control) or treated red haricot beans (test diets) with or without multienzyme. Red haricot beans used in the experiment were raw, fermented or soaked and oven heated. Feed intake and weight gain were measured weekly. On the 28th day one bird per cage was sacrificed to measure pancreas weight which was expressed as a percentage of the body weight. The results indicated that fermentation and oven heating had little effect on red haricot bean Anti-nutritive Factors (ANFs). Enzyme supplementation also had no effect on red haricot bean ANFs. However, increased feed intake on enzyme addition is indicative of enzyme effects on dietary Crude Fibre (CF).

Key words: broiler performance, enzyme, fermentation, oven heating, red haricot.

4.2 Introduction

The large and diverse legume family has given the world an abundance of crops for food, forage, fibre, feed and ornamental uses. Legumes were among the earliest crops to be cultivated throughout the world by man and have protein content raging from 17% - 25%, which is nearly twice that of cereals. The nutritional value of grain legumes depends upon the anti-nutritive factors contained as well as natural toxicants such as cyanogens. The negative nutritive components reduce their protein digestibility, bioavailability and its general nutritive value. Feeding raw legumes to chickens generally results in lower growth rate and reduced feed efficiency compared with feeding processed legumes. However, each legume produces a different response after processing and feeding to poultry (Wiryawan and Dingle, 1998). This has led to reduced utilization of legumes in nonruminant diets. Several processing techniques including heating, soaking, germination, fermentation and enzyme supplementation have been utilized to reduce or eliminate antinutritive factors and toxicants in legumes. It has been established that when legumes are properly processed their nutritive value increases greatly.

The aim of this study was to investigate the potential of heating; fermentation and multienzyme supplementation as bean processing methods to reduce ANFs and improve utilization by broilers. Effect of combining the traditional processing methods (cooking and fermentation) with enzymes was also studied.

4.3 Materials and methods

The experiment was conducted at Kenya Agricultural Research Institute (KARI), Naivasha

4.3.1 Processing and feed preparation

Beans

Two hundred and Forty kilogrammes (240kg) of red haricot beans were bought from Naivasha Municipal market, cleaned of all foreign materials and divided into three batches of 80 kg each. The first batch was ground to pass through a 2 mm sieve. Using EM technology of making bokashi as described in the literature review page 49, the ground beans were ensiled in 1000 mm gauge polythene tubes for 14 days. On the 14th day, the fermented flour was removed from the tubes and oven dried at 60^{0}C. The dried material was then ground to break the clumps. The second batch of beans was soaked overnight (24 hours). After draining the soaking water the beans were heated in an oven at 100^{0}C for 20 minutes. The grains were dried for 24hrs at 60^{0}C and ground to pass through a 2mm sieve. The third batch of red beans was simply ground to pass through 2mm sieve and used in diets without further processing.

Enzymes

The multienzyme used in this experiment is a product of Alltech International, Allzyme SSF, produced from fungi called *Aspergillus niger*. The enzyme is produced through solid state fermentation of wheat bran by *Aspergillus niger*. It has activities for many enzymes:

Amylase, Cellulase, Phytase, Xylanase, Beta-glucanase, Pectinase and Protease. The multienzyme was purchased from Altech International sales office in Nairobi.

4.3.2 Experimental diets

Seven isonitrogenous broiler starter diets were formulated to contain 18% CP and 2800 Kcal ME per kilogramme. The seven treatments were as follows:

T1 Maize germ-soy bean cake control diet or maize-SBM.

T2 Maize germ-red haricot (Raw)

T3 Maize germ-red haricot (Raw + Enzyme)

T4 Maize germ-red haricot (Oven heated)

T5 Maize germ-red haricot (Oven heated + Enzyme)

T6 Maize germ-red haricot (Fermented)

T7 Maize germ-red haricot (Fermented + Enzyme)

Table 4.1 Composition of the experimental diets

Ingredients (%)	**Diets**						
	T1	T2	T3	T4	T5	T6	T7
Maize Germ	69.3	48.30	48.28	48.30	48.28	48.30	48.28
SBM	16	-	-	-	-	-	-
Red haricot	-	37	37	37	37	37	37
Fish Meal	10	10	10	10	10	10	10
Limestone	1.7	1.7	1.7	1.7	1.7	1.7	1.7
DCP	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Enzyme	-	-	0.02	-	0.02	_	0.02
Premix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated Analysis							
ME (Kcal/kg)	2850.34	2740.16	2740.27	2780.80	2760.52	2750.26	2740.39
CP (%)	18.46	18.26	18.38	18.45	18.35	18.64	18.33

Key

[1]Vitamins and trace minerals per Kg of the diet: Vitamin A, 6500 IU; Vitamin D3, 3000 IU; Cholin equivalents, 800 mg; Riboflavin, 5.5 mg; Pantothenic acid, 14 mg; Vitamin B12, 0.013; Folic Acid, 1.0 mg; Biotin, 0.20; Niacin, 40 mg; Vitamin K, 2.0 mg; Vitamin E, 30 IU; Thiamin, 4.0 mg; Pyridoxine, 4.0 mg; Manganese, 70 mg; Iron, 80 mg; Copper, 10 mg; Zinc, 80 mg; Selenium, 0.30 mg; Iodine, 0.4 mg.

Each diet was randomly fed to four replicate pens (10 chicks/pen) for four weeks. Feed was offered libitum. Availability of fresh clean drinking water was assured throughout the experimental period.

4.3.3 Birds, housing and management

Two hundred and eighty broiler chicks (Cobb's hybrid) of mixed sexes were purchased from a commercial hatchery. All the experimental birds were initially weighed and put on a commercial starter diet for six days. On the seventh day the chicks were randomly divided into 28 experimental units of 10 chicks each.

The experimental unit comprised of 28 pens and each pen housed 10 chicks. The broiler house was washed and disinfected by fumigation before the onset of the experiment. All experimental chicks were reared on deep litter throughout the experimental period of 4 weeks. The placement of each chick in the pen was also made at random. The temperature of the experimental room was maintained with infrared bulbs provided for each pen to keep warmth at 33±2°C during the first week of trial and then reduced by 3°C each week till it reached 25°C which was maintained for the rest of the period. Proper management practices like ventilation, sanitation etc were maintained throughout the experimental period.

4.3.4 Data collection

Production performance (weight gain, feed intake and feed conversion) were measured for 4 weeks. Feed was offered every morning and the left over feed weighed at the end of each week to determine weekly feed consumption. Weight gain was recorded weekly. Data on weight gain and feed intake was used to calculate feed conversion ratio (FCR). Mortality and general observations were also recorded during the trial. The feeding trial lasted 28 days. At the end of the experiment, three birds from each experimental treatment were picked randomly and slaughtered to record the liver and pancreas weights relative to body weight.

4.3.5 Statistical analysis

The experiment was conducted under completely randomized design. The production performance data obtained were analyzed by analysis of variance using GLM procedures of SAS 2001. The statistical model used for analysis was $Y = a + b(\text{diet}) + E$ where

Y = dependent variable

a = Y intercept

b = slope of the gradient

E = error

Differences among means were compared by Duncan’s multiple range tests at 5% probability (Duncan, 1955).

4.4 Results

4.4.1 Effects of total substitution of soybean cake with red haricot beans

Total substitution of soy bean cake with raw or processed red haricot beans in broiler diets significantly reduced weight gain and feed intake while increasing feed to gain ratio and mortality rates in broiler chicks (Table 4.2) . Chicks fed soybean cake based diet (control diet) performed significantly better than chicks fed red haricot bean based (test) diets. Pancreas weight for chicks fed on test diets was above 0.4% of bodyweight (Table 4.3) indicating pancreatic hypertrophy in all the test diets. Normal pancreas in chicken is about 0.3% of body weight (Leeson and Summers, 1997). Sizes above 0.3% are indicative of pancreas hyperactivity; an indication of some residue TIA activity. Liver sizes for chicks on test diets were not statistically different from the control diet (Table 4.2). However, there was a tendency for enzyme addition to reduce liver sizes in the test diets. Chicks fed test diets registered higher mortality rates than chicks on control diet.

Table 4.2 Effects of total substitution of soybean cake with common beans on broiler performance

Parameter	Diets							
	T1	T2	T3	T4	T5	T6	T7	SEM
Weight gain(g/wk)	218^{a}	56^{b}	54^{b}	56^{b}	52^{b}	58^{b}	56^{b}	29.29
Feed intake(g/wk)	384^{a}	216^{b}	274^{cd}	266^{cd}	248^{c}	250^{c}	288^{d}	19.17
FCR	1.76^{a}	3.86^{b}	5.01^{c}	4.75^{bc}	4.76^{bc}	4.31^{b}	5.14^{c}	0.10
Pancreas weight (%)	0.377^{a}	0.475^{ab}	0.585^{b}	0.459^{a}	0.429^{a}	0.456^{a}	0.460^{a}	0.07
Liver weight %	3.35^{a}	4.06^{a}	3.14^{a}	3.74^{a}	3.35^{a}	3.64^{a}	3.45^{a}	0.63
Mortality (%)	2.5	17.5	27.5	17.5	20.0	17.5	15	

4.4.2 Effects of enzyme supplementation on utilization of red haricot beans (raw, cooked or fermented) based broiler diets

Addition of enzymes at the rate of 0.02% to raw (T3) or processed red haricot bean based diets (T5 and T7) produced mixed results. Addition of enzymes to the diets containing raw (T3) or fermented (T7) red haricot beans improved feed intake significantly. However the improvement in intake was still significantly lower than the control diet (Table 4.3). Enzyme addition however had no significant effect on intake of diets based on cooked bean (T5) though there was a tendency towards a decrease in intake. Feed intake declined by 9% on addition of enzymes in the diets based on cooked beans.

The improved feed intake with enzyme addition in raw and fermented beans based diets did not translate into significant weight gains (Table 4.3). Addition of enzymes to the diet based on raw beans (T3) improved feed intake significantly (27%) while growth rate and feed conversion ratio declined by 3.5% and 29.7% respectively. This increased feed intake also increased pancreas size significantly by 23%. Similarly enzyme addition improved intake of diets based on fermented beans by 15% while weight gain actually reduced by 3.5%, feed conversion ratio reduced by 19.5% and pancreas size remained unchanged.

Improved feed intake implies increased rate of digesta flow due to improved digestibility. This however seems to have worsened the lectin and trypsin inhibitor problem as evidenced by increased mortality, size of pancreas and negligible growth rate. Increased digesta flow due to enzyme action may have increased chick exposure to toxins which bound and inactivated more digestive enzymes. This triggered increased pancreas activity with a consequent increase in pancreas size. This diverted more and more methionine from the body exacerbating an already existing methionine deficiency in common beans. These results agree with laboratory results of chapter three which indicated only a 22% reduction in TIA activity after solid state fermentation of common beans.

4.4.3 Effects of bean processing on broiler performance

4.4.3.1 Fermentation

Feed intake was significantly ($P<0.05$) improved by fermentation of red haricot beans (T6) compared to diet based on raw red haricot beans (T2). Chicks offered diets with fermented beans (T6) recorded higher feed intake (15.7%) and weight gain (3.6%) than chicks on diet with raw red haricot beans (T2). Feed conversion ratio tended to increase by 11.4%. Pancreas size and mortality rates were not improved by fermentation. This implies that fermented red haricot beans were as poisonous to chicks as raw beans. Chicks offered the control diet (T1) had significantly higher feed intake and weight gain than chicks fed on diets with either raw or fermented beans.

4.4.3.2 Oven Heating

Consumption of raw red haricot beans by broiler chicks significantly reduced body weight gain and feed intake compared to maize–SBM control diet. Thermal processing significantly improved feed intake (23%) compared to diet with raw beans. The increased intake had no corresponding effect on weight gain. Weight of the pancreas (relative to body weight) increased significantly in birds fed raw red haricot beans compared to maize – SBM control diet but was reversed (3.4%) on soaking and heating of the beans.

These results indicate that soaking and oven heating beans at 100^0C was partially effective in reducing ANFs as evidenced by significant increase in feed intake in addition to positive effects on pancreas weight observed in this study. However, the process was not very effective in reducing all antinutritive factors to safe levels probably due to short heating time or oven heating as opposed to boiling with water. Addition of enzymes to the diet with cooked beans reduced feed intake by 6% while improving weight gain and feed conversion efficiency by 5.3% and 9% respectively. These results imply that enzymes had positive effects on both the nutrient absorption and utilization.

4.5 Discussion

4.5.1 Effects of enzyme supplementation on utilization of broiler diets based on red haricot beans

Use of enzymes in the test diets did not improve broiler performance to the levels of control diet (T1). All the diets with enzymes supported broiler performance significantly ($p<0.05$) poorer than control diet. However addition of enzymes to the diets containing raw (T3) or fermented (T7) red haricot beans improved feed intake significantly compared to the diets T2 and T6 which had no enzymes though the improvement in intake was still significantly lower than the control diet (Table 4.2). This was not observed when the enzymes were added to diet with soaked and oven heated red haricot beans (T4 and T5). The improved intake however did not translate into improved weight gain or FCR. The results obtained in this study are in agreement with results of others. Farhoomand and Poure (2006) reported that addition of 0.05% enzyme (*Vitas aim x* manufactured by Vita international Ltd Vita A/S Tehran Iran; enzyme activities per gram of crude product was 3000 EPU) to diets containing 20% whole raw peas had no beneficial effect. Irish et al. (1993) and Marsman et al. (1997) have reported no improvement in performance of 1-25 day old chicks fed SBM diets supplemented with enzymes. Ma and wang (2010) reported that single or mixture of protease enzymes had reduced lectin activity by 21% in raw soybeans while heating reduced lectins activity by 53%. Enzymes cannot denature and deactivate raw lectins due to the compact molecular structure, which may inhibit the access of enzymes to the cleavage site.

Increased digestibility and digester flow may therefore serve to increase the negative effects of ANFs due to their increased flow and exposure in the gastro-intestinal tract. This would call for high rates of enzyme addition to increase chances of some enzymes being spared to act on the legume ANFs or pretreatment of legumes before inclusion in the rations. In this study addition of enzymes to diet with raw beans increased mortality rates from 17.5% to 27.5%. Enzymes also increased feed intake significantly by 27% without improving weight gain. This could imply partial effects on some ANFs but not enough to improve absorption and utilization. Increased mortalities and larger pancreas on addition of enzymes also points to increased activity of pancreas. Pancreas size in birds taking raw beans without enzyme was larger than control by 26% while on addition of enzymes the difference increased to 55%. This points to increased chick exposure to higher quantities of TIA with increase in feed intake implying that increased feed intake merely exposed the chicks to higher quantities of TIA and possibly lectins. This could be due to low levels of enzyme supplemented (0.02) and resultant dilution effect by other ingredients leading to little effect on TIA (Thorpe and Beal, 2001) or that action of enzymes on NSP with no effect on TIA may have exposed birds to higher of TIA. It is more likely that enzymes reduced the levels of soluble fraction of dietary fibre with little or no effect on the insoluble fraction. This led to increased digester flow and resultant increase in feed intake with no resultant increase in performance. Soluble fibre increases intestinal transit time, delays gastric emptying, increases pancreatic secretion, and slows absorption (i.e. reduces feed intake) whereas insoluble fibre decreases transit time and enhances water holding capacity i.e. increases flow rate and feed intake (Montagne et al., 2003). Enzymes improve feed utilization when viscous cereals (soluble fibre) are the major source of energy. Non-starch polysaccharides, specifically the soluble fraction, have a negative impact on digestion and absorption of nutrients in poultry. The mechanisms by which NSP reduce broiler performance are not well understood. Soluble NSP increase digesta viscosity and reduce the accessibility of enzymes to starch, protein, and lipids of the diet. Added enzymes therefore improve bird performance by increasing nutrient digestibility and feed intake (Mateos et al., 2006). In this study, increased digesta flow exposed birds to higher levels of lectins and TIA as evidenced by increased pancreatic weights and mortality rates.

It can therefore be concluded that enzymes may have acted on the soluble fibre fraction of the diet leading to increased rate of digesta flow but these enzymes had little or no effect on TIA and lectins either due to dilution effects or failure to completely neutralize them.

4.5.2 Effects of bean processing on broiler performance

4.5.2.1 Fermentation

Diets containing fermented red haricot beans (T6 & T7) performed significantly poorer than control diet (T1). However, compared to the diet with raw red haricot beans (T2) the T6 and T7 diets supported a significantly higher feed intake though this did not translate into significant improvements in weight gain or FCR. This is indicative of some beneficial effect of fermentation on red haricot bean ANF's. Results presented elsewhere in this report (Chapter Three) show a 94% and 22% reduction of lectins and TIA respectively on fermentation of red haricot beans for 14 days.

These results agree with the work of Martín-Cabrejas et al. (2004) who reported that fermentation treatment significantly decreased the soluble dietary fibre (SDF) content, but had minimal effect on the insoluble dietary fibre (IDF) content of the processed beans. The authors observed that the fermentation microbes acted on the bean cell wall, disrupting the protein−carbohydrate integration, thus reducing the solubility of dietary fibre (DF). Reduction of SDF improves feed intake, digesta flow and digestibility (Montagne et al., 2003; Mateos et al., 2006). In this study birds fed diet with fermented beans had a significantly higher feed intake than birds fed diets with raw beans.

Ikemefuna et al. (1991) reported that fermentation reduced cyanide in pre-soaked seeds but cooking and fermentation reduced cyanide in soaked seeds to safe levels. They further reported that cooking and fermentation synergistically reduced tannins. Cooking and fermentation broke down tannin-enzyme and protein-tannin complexes and released free tannins which subsequently leached out. Ikemefuna et al. (1991) concluded that fermentation appeared to have beneficial effects as a method of processing. Combinations of cooking and fermentation improved the nutrient quality and reduced the anti-nutritional factors inherent in germinated cereal products to safe levels much greater than any of the other processing methods tested. Fermentation of *Phaseolus vulgaris* (black bean) seed with *Lactobacillus casei* and *Lactobacillus plantarum* inocula produced a 63.35% decrease in soluble fibre and 88.6% for raffinose. When cooking under atmospheric pressure was applied to the fermented samples, a significant decrease in the trypsin inhibitors as well as an increase in the *in vitro* and *in vivo* digestibility of the beans occurred. The results demonstrate that the lactic acid bacteria used as inoculant in fermentation can lead to a functional feed with improved nutritional quality (Granito and Alvarez, 2006). Generally, adequate heat processing inactivates the trypsin and chymotrypsin (Dipitero and Liener, 1989; Osman et al., 2002) while heat stable compounds in cereals and legumes such as tannins and hydrates are easily removed after germination (Reddy et al., 1985) and fermentation (Osman, 2004).

Protease inhibitors in diets led to formation of irreversible trypsin enzyme-trypsin inhibitor complex, causing a trypsin drop in the intestine. This decreases digestibility of diet protein, resulting to slower animal growth. The organism in this situation increases the secretory activity of the pancreas leading to increased pancreas size (Chumnei et al., 2010). It appears therefore that fermentation did reduce ANFs as evidenced by reduction in pancreas size and increase in feed intake after fermentation. This also agrees with the results of chapter three which indicated a partial reduction of TIA on fermentation of beans.

4.5.2.2 Heat treatment

Diets containing soaked and oven heated red haricot beans (T4 & T5) performed significantly ($p<0.05$) poorer than control diet (T1) on all parameters tested except pancreas weight.

However compared to the diet with raw red haricot beans (T2) the T4 and T5 diets supported a significantly higher feed intake though this did not translate into significant improvements in weight gain or FCR. This is again indicative of some beneficial effect of soaking and oven heating on red haricot bean ANF. These results agree with the results of Antoine (2009) who compared the growth rates of broiler chicks fed diets containing either 30% heat-treated or 30% raw versions of the three bean cultivars for 21 days. The bean flour used was dry toasted in a layer 3 cm. deep at 130º C for 35 minutes. The author reported that chick growth was improved by the heat treatment of Great Northern white beans; while heat treatment demonstrated little or no impact on the birds fed pinto or small red beans.

Different heat treatment methods therefore have varying effects on common bean ANFs. Teguia and Fru (2007) reported that inclusion at 15% soaked or soaked and oven heated common beans in broiler diets had significantly poorer performance compared to control or diets with extruded (145^0C) common beans. This clearly indicates ANF deactivation is dependent on cooking temperature, presence or absence of water and duration of heating. Ramakrishna et al. (2008) have reported that roasting was only effective in reducing the phytic acid and TIA while boiling and pressure cooking were effective in reducing total polyphenols, tannins and TIA. Boiling and pressure cooking were effective in reducing total polyphenols, tannins and TIA. A further loss of 11 per cent in the TIA was noticed by pressure cooking compared with boiling. Farhoomand and Poure (2006) reported that autoclaving (20 minutes at 121^0C) cooking (20 minutes in boiling water) and dehulling of yellow peas improve feed conversion rate and increased body weight gain in broilers though autoclaving gave the better results. Ogundipe (1980) reported that moist cooking of soybeans gave better performance in broilers than other processing methods. Generally dry heat treatment is not as effective as moist heat in deactivating ANFs. Valverde et al. (1997) reported a TIA reduction of 69% and 100% on dry heating and boiling Faba beans respectively for 35 minutes. This could explain the situation observed here whereby significant increase in feed intake did not translate into significant improvement in weight gains or pancreas size. This therefore implies that despite increased intake, feed absorption and utilization remained low while exposure to ANFs remained high. This supports the conclusion of Ramakrishna et al. (2008) that roasting is not effective in reducing TIA and soluble fibre.

The poor performance of chicks fed oven heated beans in this study could be due to effects of residual and heat stable ANFs. Sathe and Salunke (1984) reported that trypsin inhibitor in beans, cowpea, and black gram was resistant to dry heat. Heat resistance of trypsin inhibitor has also been reported for bean flour processed at 97°C for 30 minutes (Carvalho and Sgarbieri, 1997). In addition to heat stable protease inhibitors, residual TIA is attributed to the presence of non-protein components such as browning substances (resulting from Maillard reactions), phytate, phenolics, and a relative concentration of fibre which could give rise to residual inhibitor activity after dry heat processing. Polyphenolic compounds constitute majority of residual TIA in heated winged beans (Deshpande, 1992).

4.6 Conclusion

From the results of this study it can be concluded that:

1) Enzyme supplementation, soaking and heating or fermentation are not effective in reducing the negative effects of red haricot beans to safe levels in broiler diets

2) Enzyme supplementation, soaking and heating or fermentation seem to have partial effects on red haricot bean composition as evidenced by a significant increase in feed intake after the treatments compared to untreated beans.

CHAPTER FIVE
5. PARTIAL SUBSTITUTION OF SOYBEAN WITH RED HARICOT BEANS IN BROILER DIETS: EFFECTS OF PROCESSING, PROBIOTICS AND ENZYMES ON PERFORMANCE

5.1 Abstract
Effects of boiling soaked red haricot beans, multienzyme supplementation or probiotic supplementation on broiler performance was investigated in a 28 day trial. Enzyme supplement was added to a complete ration at 0.2% while probiotics were provided in drinking water at the rate of 50mls EM per 20 litres of water (a ratio of 1:400 or 2.5mls/l). Three hundred and twenty (320) seven day old broiler chicks were distributed to eight diets in a completely randomized design to give four cages per diet (10 chicks per cage). The diets contained maize, fish meal, SBM and variously treated red haricot beans (test diets). Red haricot beans were used in the raw, fermented or soaked and boiled in water for 30 minutes. Feed intake and weight gain were measured weekly. On the 28^{th} day one bird per cage was sacrificed to measure pancreas weight which was expressed as a percentage of body weight. Birds fed diets containing 20% soaked and cooked red haricot beans performed similar to birds fed Maize-SBM control diet. Addition of probiotics or enzymes to maize-cooked beans diets significantly ($p<0.05$) depressed intake compared to maize – SBM control diet. Diets with 20% raw or fermented beans performed significantly ($p< 0.05$) poorer than the diets with maize- cooked bean or maize – SBM control diet. Pancreas size in birds fed maize-cooked bean diet was not significantly different from maize-SBM control diet. It is concluded that soaked and boiled red haricot beans can be included in broiler starter diets at the rate of 20% without any adverse effects on performance.

Key words: broiler performance, enzymes, probiotics, red haricot beans

5.2 Introduction
Commercial poultry feed industry is largely dependent on the conventional protein sources mainly; soybean meal for practical diet formulations. Soybean meal is produced in Kenya in very small amounts hence most of the meal used in feed manufacture is imported. This results in a high feed production cost. The ban by the European Union and America on the use of animal by-products in ruminant diets makes the situation more critical for the poultry

industry. Therefore, evaluation of alternative protein ingredients, which are locally available, economical, and can be used as substitutes for conventional protein sources, is necessary.

The interest of using grain legumes such as common beans, faba beans, lupins and peas as an alternative to conventional protein sources has been increasing (Wiryawan, 1997; Li et al., 2006). These legume seeds not only offer a valuable source of protein, but also provide energy due to their high starch and oil contents (Diaz et al., 2006). However, these legume seeds also contain variable amounts of anti-nutrititive factors such as non-starch polysaccharides, tannins, lectins and protease inhibitors which can reduce nutrient digestibility, performance and adversely affect digestive tract development (i.e. pancreatic and small intestinal enlargements) in birds. Thus, for the maximal use of these legume meals, it is important to know how processing and feed additives influence their utilization in practical broiler diets whilst still maintaining performance. Although considerable research has been carried out on the suitability of grain legumes either partly or fully replacing soybean meal in broiler diets, data on inclusion levels are contradictory (McNeill et al., 2004; Olkowski et al., 2005).

Thus, the present study was carried out in order to examine the effects of feeding diets containing 20% of raw or processed red haricot beans, with or without additives, on the performance of broilers. This inclusion level was chosen on the basis of available published data. The additives used were Allzyme SSF enzyme and effective microorganisms.

5.3 Materials and methods

The experiment was conducted at Kenya Agricultural Research Institute (KARI) poultry unit, Naivasha.

5.3.1 Ingredients processing

Beans

Two hundred and forty (240) kilogrammes of red haricot beans were bought from Naivasha municipal market, cleaned of all foreign materials and divided into three batches of 80 kg each.

Fermenting

The first batch was ground to pass through a 2 mm sieve. Using EM technology of making bokashi as described in chapter two, the ground beans were ensiled in 1000 mm gauge polythene tubes for 14 days. On the 14^{th} day, the fermented flour was removed from the tubes and oven dried at 60^0C for 24 hours. The dried material was then ground to break the clumps and used to make diets based on fermented beans.

Boiling

The second batch of beans was soaked for 24 hours and cooked in boiling water for 30 minutes. The grains were then dried in an oven at 60^0C for 24 hours and ground to pass through 2mm sieve. The flour was used to make diets based on cooked beans.

Raw beans

The third batch of red beans was simply ground to pass through 2 mm sieve and used in diets with raw beans without further processing.

Enzymes

The mult-enzyme used in this experiment was a product of Alltech International, Allzyme SSF, similar to the one used in the previous experiment.

The probiotic used was effective imcroorganisms (EM) bought from an Agrovet shop in Naivasha town.

All the feed ingredients and enzymes were procured locally.

5.3.2 Birds and housing

Three hundred and twenty (320) day-old broiler chicks were obtained from Kenbird hatcheries in Naivasha Town. The birds were fed on commercial broiler starter diets for seven days after which they were randomly allocated to thirty two cages (10 birds per cage). Each cage was electrically heated with an infrared bulb so as to maintain a temperature of 32-35^oC in the brooder house. The cage floors were covered with wood shavings litter.

5.3.3 Experimental diets

Eight isonitrogenous and isocaloric broiler starter diets were formulated to contain 22% CP and 3000 kcal of energy per kilogramme (Table 5.1). There were four types of diets:

i) Maize-soy bean cake control diet (D1)

ii) Maize – soaked and cooked red haricot beans (D8)

iii) Maize- red haricot beans (raw, soaked and cooked or fermented) with enzyme (diet D5, D6 and D7).

iv) Maize- red haricot beans (raw, soaked and cooked or fermented) with probiotic (D2, D3 and D4)

Table 5.1: Composition of diets

Ingredients	**Diets**							
Additive	Control	Probiotic			Enzyme			None
Bean processing	None	Raw	cook	ferment	raw	cook	ferment	Cook
Maize	48.80	37.14	37.14	37.14	37.12	37.12	37.12	37.12
SBM (44%)	23.00	20.66	20.66	20.66	20.66	20.66	20.66	20.66
Red Haricot	-	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Maize Gluten	14.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Fish meal	5.00	3.0	3.00	3.00	3.00	3.00	3.00	3.00
Vegetable Oil	4.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Enzyme	-	-	-	-	0.02	0.02	0.02	0.02
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated analysis								
Crude protein (%)	21.65	21.64	21.64	21.64	21.58	21.58	21.58	21.58
AvailableME (Kcal/kg)	3023.3	2968.4	2968.4	2968.40	2967.7	2967.7	2967.73	2967.7

Key

* Vitamins/kg of diet: Vitamin A, 6500 IU; Vitamin D3, 3000 IU; Choline equivalents, 800 mg; Riboflavin, 5.5 mg; Pantothenic acid, 14.0 mg; Vitamin B_{12}, 0.013 mg; Folic acid, 1.0 mg; Biotin 0.20 mg; Niacin, 40.0 mg; Vitamin k, 2.0 mg; Vitamin E, 30.0 mg; Thiamine, 4.0 mg; Pyridoxine, 4.0 mg; Manganese, 70mg; Iron, 80 mg; Copper, 10 mg; Zinc, 80 mg; Selenium, 0.30 mg; Iodine, 0.40 mg.

Enzymes were included in the diets at the rate of 200g/ton as recommended by the manufacturer while probiotics were supplied together with drinking water at the rate of 50mls EM per 20 litres of water (a ratio of 1:400 or 2.5mls/l). Fresh EM: water mixture was prepared daily. Each of the eight dietary treatments was randomly assigned to four cages which contained ten chicks each. The diets were offered *ad libitum* from day 7 to day 35. Water was freely available throughout the trial.

5.3.4 Data collection

Body weights and feed intake were recorded at weekly intervals throughout the trial. The weight gain was calculated as the difference between initial and final weights per bird. Feed intake was calculated as the difference between total feed provided per week minus the feed leftovers. Feed conversion ratio was calculated as feed intake divided by weight gain. Bird mortality was recorded daily and dead birds were removed and weight was recorded. Fresh faeces were collected from the cages and faeces for each treatment were pooled, mixed and a sample taken for wetness determination. Excreta wetness was determined by drying the samples at 100 ^{0}c for 24 hours. On day 35, three birds per treatment were selected randomly, weighed and sacrificed by cervical dislocation. The pancreas and liver were removed and weighed.

5.3.5 Statistical analysis

Cage means were used to derive performance data. For pancreas and liver analysis individual birds were considered as the experimental units. All data were subjected to analysis of variance using the General Linear Model procedure of SAS (2001). The statistical model for analysis was

$Y = a + b\,(\text{diet}) + E$

Where Y = dependent variable

a = Y intercept

b = slope of the gradient

E = error

Differences were considered to be significant at $P<0.05$ and significant differences between means were separated by the Duncan's multiple range test (Duncan, 1955).

5.4 Results

5.4.1 Effects of diet on broiler performance

The average performance of the broilers as affected by the presence of red haricot in the diet, additives used and the processing method applied to detoxify the beans are summarized in Table 5.2. In general, performance of the birds fed with diets containing 20% common beans (replacing 50% of soybean protein) varied based on processing method applied and presence or absence of probiotics and enzymes.

Table 5.2 Performance of broilers fed maize-common bean diets with addition of EM and/or enzyme

Parameter	Diets								SEM
Additive[1]	Control	EM	EM	EM	Enzyme	Enzyme	Enzyme	None	
Processing	None	Raw	Cook	Ferment	Raw	Cook	Ferment	Cook	
WG/wk (g)	230^{a}	123^{c}	199^{b}	116^{c}	120^{c}	201^{b}	111c	213^{ab}	9.59
FI/wk (g)	445^{a}	338^{c}	425^{ab}	330^{c}	340^{c}	403^{b}	324^{c}	441^{a}	16.97
FCR	1.93^{a}	2.76^{c}	2.14^{b}	2.86^{c}	2.83^{c}	2.00^{ab}	2.91^{c}	2.08^{ab}	0.13
Liverwt %	3.26^{a}	3.15^{a}	2.75^{ab}	2.85^{ab}	3.37^{a}	2.73^{ab}	3.22^{a}	2.43^{b}	0.24
Pancreas wt %	0.43^{b}	0.60^{ab}	0.52^{ab}	0.58^{ab}	0.63^{ab}	0.55^{ab}	0.69^{a}	0.45^{b}	0.08
Wetness %	50	61.5	62.35	67.5	63.3	62.5	72.2	70.83	

Key

[1]additives: EM= Effective Micro-organisms; Enzyme =Allzyme SSF

[ab]Means with different superscripts in the same row were significantly different ($p < 0.05$)

Feed intake was significantly depressed by inclusion of 20% raw or fermented common beans in broiler diets. However, inclusion of a similar amount (20%) boiled common beans in the diet supported similar broiler performance with the control diet though there was a tendency towards a depressed weight (Table 5.2). Feed conversion ratio results showed similar trends with feed intake and weight gain. Control diet and diets with 20% soaked and cooked red haricot beans had significantly lower FCR compared to diets with raw or fermented common beans.

5.4.2 Effects of bean processing method on broiler performance

Soaking and cooking red haricot beans for thirty minutes resulted in a performance of broilers similar to the control diet. Birds fed diets containing 20% cooked beans had a similar performance with birds fed maize–SBM control diet. Feed intake, weight gain, feed conversion efficiency and pancreas weight were not significantly different between control diet and diet with cooked beans. Chicks fed a diet with cooked beans had similar pancreas weight with control diet (0.45 and 0.43 respectively). Generally broilers fed diets with 20%

raw or fermented common beans recorded significantly poorer performance compared to broilers on control diet or cooked beans based diets. The pancreas weight for birds fed these diets also tended to become larger compared to control or diets with cooked beans. This can be attributed to high ANF levels in the raw and fermented diets. This confirms that solid state fermentation has little effect on common bean ANFs.

Inclusion of additives (probiotics or enzymes) to the diet with cooked beans in this experiment tended to increase Pancreas size of the birds (Table 5.1). This implies increased exposure of birds to residual TIA. Defang et al (2008) have reported that boiling black common beans for 30 minutes does not completely eliminate ANFs.

5.4.3 Effect of additives on utilization of diets with raw or processed red haricot beans

Probiotics

Addition of probiotics to the diet with cooked red haricot beans tended to decrease both weight gain (6.5%) and feed intake (3.6%) compared with maize– cooked bean diet without additives. Feed conversion Ratio also reduced by 3% (Table 5.2). Addition of probiotics to cooked bean diet also increased pancreas weight by 16% (Table 5.1). This indicates no beneficial effects of feeding probiotics to broilers. Addition of probiotics to diets with raw or fermented common beans had also no beneficial effect on any of the performance parameters measured.

Enzymes

Addition of enzyme to the diet with cooked red haricot beans significantly decreased feed intake without affecting weight gain though there was a tendency to decrease. Though enzymes significantly decreased feed intake, the pancreas weight of the birds actually increased by 22% while feed conversion ratio increased by about 4%.

5.5 Discussion

5.5.1 Effect of diets on broiler performance

Diets with 20% soaked and cooked red haricot beans supported similar performance with the standard maize - SBM control diet. However, diets containing 20% raw or fermented beans had significantly poorer performance than the control ($p < 0.05$). Similar results have been reported by Defang et al. (2008) who reported that a diet containing 14% black common beans or cowpeas supported similar broiler performance with maize- SBM control diet with a tendency to depression. Teguia and Fru (2007) also reported that a diet with 15% extruded (145^0C) common beans supported similar performance to the control but with a tendency to depression. The depressive effect on weight gain induced by soaked and boiled red haricot beans observed with broilers could be due to the inability of the treatment method to properly deactivate the ANFs (protease and lectins inhibitors) present in the common bean that are responsible for weight depression (Defang et al., 2008). Fermentation as a processing method of red haricot beans appear to have had little or no effect on the ANF's as indicated by similar performance of birds fed raw or fermented beans based diets.

5.5.2 Effect of bean processing method on broiler performance

Soaking and Cooking

Soaking and cooking red haricot beans for thirty (30) minutes resulted in a performance of broilers similar to the control diet. These results agree with Farhoomand and Poure (2006) who reported that autoclaving, cooking and dehulling of yellow peas improved feed conversion efficiency and increased body weight gain in broilers. Ofongo and Ologhobo (2007) performed a similar experiment where kidney beans were cooked for one hour in boiling water and used to replace 50% of SBM protein. The beans formed 27% of the diet. The authors reported similar performance for control diet and test diets. The results also agree with Emiola et al. (2007) who concluded that, aqueous heated kidney bean meal can be used to replace 50% protein supplied by soybean meal in broiler starter and finisher diets without any adverse effect on the performance and the internal organs. This also agrees with Wiryawan (1997) who stated that among the potential sources of vegetable proteins, leguminous grains such as *Phaseolus vulgaris* (common beans) can serve as alternatives to fat-extracted soybean meal because they have similar amino acid profiles. But this can only happen after reduction of ANFs to acceptable levels (Defang et al., 2008).

The absence of significant difference for weight gain, feed intake, feed conversion ratio and pancreas weight between the birds on maize – cooked red haricot diet and maize – SBM control diet is a confirmation that cooking beans for 30 minutes in boiling water as performed in the present study, deactivates common bean ANFs to safe levels. Herkelman et al. (1991) have also reported a pancreas weight of 355mg/100g and 352 mg/100g respectively for chicks fed soybean meal or full fat soybeans heated at 121^0C for 40 minutes. However, there may be some residual ANFs capable of affecting performance under certain conditions.

5.5.3 Effect of additives on utilization of diets with raw or processed red beans

Enzymes

Enzymes significantly ($p<0.05$) reduced feed intake of diets based on cooked red haricot beans while maintaining weight and FCR. Addition of enzymes also tended to increase pancreas weight. This agrees with our results presented in chapter four that enzymes actually tended to improve utilization of diets based on cooked red haricot beans. Diets based on raw or fermented beans resulted in significantly ($p<0.05$) poorer broiler performance than control diet. This again agrees with Noreen, and Salim (2008) and Kakade et al. (2010) who have reported that unheated navy bean trypsin inhibitor is resistant to enzymatic attack due to the stability of the molecule produced by a large number of disulphide bonds but heating causes an unfolding of the molecule resulting in the exposure of peptide bonds susceptible to enzymatic cleavage.

Reports that have utilized predominantly corn-soy based diets in enzyme research have suggested improved broiler performance on addition of carbohydrase/proteases and phytase in broiler diets. Cowieson and Adeola (2005) investigated the additive effects on performance of adding a cocktail of xylanase, amylase and protease or phytase in a diet containing 46.8% corn, 15% rye, and 33% soybean meal as primary raw materials. The authors in that study reported that individual additions of xylanase, amylase and protease mix or phytase improved BWG by 7% and 6.2%, respectively. However, the combination of all of them improved BWG on the diet by 14%, which suggested an additive response to the individual enzymes. The FCR followed a similar trend showing respective improvements of 8.4% and 7.3% from either xylanase, amylase and protease mix or phytase and a 10.4% improvement when these enzymes were combined.

However, Cowieson et al. (2006b) have also reported that the responses of broiler chicken in trials that evaluated the addition of exogenous carbohydrase and proteases to corn-soy-based diets were variable, and difficult to predict. These authors attributed the variable enzyme responses in these studies to a lack of understanding of the nature of the substrate contained in the feed and the subsequent enzyme effects that, in-turn, led to the creation of nutrient imbalances, rather than a failure of the enzyme to modify its substrate (Cowieson et al., 2006b). This is supported by prior evidence by Collins et al. (1998) who reported that even maize, as an ingredient was potentially as variable as wheat. The large variation in the nutritive value of maize was partly attributed to variability in its chemical composition, as well as the presence of small, albeit variable quantities of anti-nutritive factors such as soluble and insoluble NSP, phytate, trypsin inhibitors and lectins. Therefore, the variability in the nutritive value of maize may affect the subsequent response to enzymes expected depending on the physiochemical properties of the maize utilized in the diet.

In this study, broilers on maize-cooked red haricot beans diet had similar performance to maize –SBM diet. However, addition of enzymes to the diet with cooked red haricot beans significantly ($p<0.05$) reduced feed intake while maintaining broiler performance though with a tendency to decrease weight gain and increase pancreas weight (Table 5.2). This clearly indicates that the enzyme mix affects the substrate digestibility and nutrient utilization. This agrees with Cowieson et al. (2006b) who stated that enzymes modify the substrate even though the results are not necessarily positive. Similar results were obtained in chapter four of this thesis. In that experiment, enzymes significantly increased feed intake while reducing weight gain for diets with raw and fermented red haricot beans. In this study, addition of enzymes to diets with raw or fermented red haricot beans resulted in significantly ($p<0.05$) poorer broiler performance than broilers fed on control diet or diet soaked and cooked red haricot bean. This further confirms that the enzymes have little or no effect on undenatured lectins and trypsin inhibitors.

The nutrient contributions from individual additions of both NSP or carbohydrase/protease enzyme cocktails and phytase have in all cases been shown to be variable and greatly dependent on the nature and amount of the raw material substrate in the diet. Therefore a particular outcome cannot be expected from these enzymes in diet formulation and their inappropriate use may end up creating nutrient imbalances and contribute to increased toxicity. This may be the reason for inconclusive effects of some studies that evaluated exogenous enzymes in diets.

Conclusion

Data obtained in this study therefore:

1. Supports the utilization of soaked and cooked red haricot beans in partial substitution of soybean as an effective protein source in broiler diets.

2. Enzymes and probiotics in general were not effective in improving utilization of diets based on raw, cooked or fermented common beans.

CHAPTER SIX

6. OPTIMUM INCLUSION LEVEL OF BOILED RED HARICOT BEANS IN BROILER STARTER DIETS

6.1 ABSTRACT

Effects on broiler performance of feeding diets containing various levels of red haricot beans boiled for 30 minutes were evaluated in a 28 day trial. Two hundred and forty (240) seven day old broiler chicks were distributed to six diets in a completely randomized design to give four cages per diet (10 chicks per cage). The diets contained maize, fish meal, soyabeanmeal and boiled red haricot beans (test diets) at 0, 15, 20, 25, 30 and 35% levels in the diet. Feed intake and weight gain were measured weekly. On the 28th day one bird per cage was sacrificed to measure pancreas weight which was expressed as a percentage of body weight. Birds fed diets containing 20% red haricot beans in the diet (50% red haricot bean protein replacing SBM protein) performed similar to birds fed Maize-SBM control diet. Diets with higher proportion of beans performed poorer than the control.

Key words: broiler performance, common beans, diets, soybean meal

6.2 Introduction

Heat treatment has proved most effective in reducing trypsin inhibitors and lectin activity in soybean (Marsman et al., 1997) and common beans (chapter three). Unheated legume protease inhibitors are resistant to enzymatic attack due to the stability of these molecules as a result of large numbers of disulphide bonds that they contain (Noreen and Salim, 2008). The heat causes an unfolding of the molecule resulting in the exposure of peptide bonds susceptible to enzymatic cleavage. The extent of inactivation is however dependent on heating time and intensity of heating. In chapter three of this work it has been shown that cooking red haricot beans in boiling water for 30 minutes is adequate to reduce ANFs to safe levels. Diets based on red haricot beans cooked for 30 minutes and included at 20% of the diet (substituting 50% of the SBM protein) supported similar performance in broilers as in maize-SBM control diet. Therefore it can be concluded that adequate heat and time inactivates the trypsin, chymotrypsin and lectins in red haricot beans. This study was designed to identify optimum inclusion levels of boiled red haricot beans in broiler starter diets.

6.3 Materials and methods

The experiment was conducted at Kenya Methodist University Farm in Meru.

6.3.1 Materials processing

Beans

One hundred and twenty five kilogrammes (125 kg) of red haricot beans were bought from Meru municipal market and cleaned of all foreign materials before cooking. The beans were immersed in boiling water and cooked for 30 minutes. The cooking beans were constantly turned to ensure even cooking. After cooking the beans were then drained and spread to dry for three days under the sun. The dry beans were then ground to pass through 2mm sieve. The flour was later used to make diets based on boiled beans.

Birds and housing

Two Hundred and forty (240) day-old broiler chicks (Cobb hybrid) were obtained from Kenchic hatchery through their agent in Meru Town. The birds were fed on a commercial broiler starter diet for seven days after which they were randomly allocated to twenty four cages (10 birds per cage). Initial weight of the birds per cage was recorded. Each cage was electrically heated with an infrared bulb so as to maintain a temperature of 32-35^{o} C in the brooder house. The cage floors were covered with wood shavings.

6.3.2 Experimental diets

Six isonitrogenous and isocaloric broiler starter diets were formulated to contain 20% CP and 2900 Kcal of energy per kilogramme (Table 6.1). The diets contained 0, 15, 20, 25, 30 and 35% red haricot beans replacing 40, 50, 60, 70 and 80 percent soybean protein respectively. Each of the six dietary treatments was randomly assigned to four cages which contained ten chicks each. The diets were offered *ad libitum* from day 7 to day 35. Fresh feed was weighed and provided each morning while the remains were pooled and weighed weekly. Fresh water was provided daily throughout the trial.

6.3.3 Data collection

Body weight and feed intake were recorded at weekly intervals throughout the feeding trial. The weight gain, feed intake and feed conversion ratio per bird per week were calculated. Weight gain was calculated as the difference between initial and final weight per chick divided by the number of weeks. Feed intake was also calculated as a weekly average per bird per week. Bird mortality was recorded daily and dead birds were removed and weight of feed and birds was recorded. On day 35, three birds per treatment were selected randomly, weighed and slaughtered. The pancreas were removed and weighed. The pancreas weight was expressed as a percentage of the bird weight. Pancreas size is propotional to body weight.

6.3.4 Statistical analysis

Treatment means were used to derive performance data. For pancreas, individual birds were considered as the experimental units. All data were subjected to analysis of variance using the SPSS version 22. The statistical model for analysis was Y = a + b (diet) + E Where:

Y = dependent variable what exactly was this?

a = Y intercept

b = slope of the gradient

E = errorDifferences were considered to be significant at $P< 0.05$ and significant differences between means were separated by Duncan's multiple range test (Duncan, 1955).

6.4 Results

6.4.1 Proximate and chemical composition of the diets

The proximate and chemical composition of the diets used in the experiment are shown in table 6.1

Table 6.1 Gross composition of the experimental diets (As fed)

Ingredients (%)	**Diets**					
SBM replacement level (%)	**0**	**40**	**50**	**60**	**70**	**80**
Maize	63	58	53	53	48	48
SBM	26	16	16	11	11	6
Red haricot	0	15	20	25	30	35
Fish meal	8	8	8	8	8	8
DCP	0.3	0.3	0.3	0.3	0.3	0.3
Limestone	2.2	2.2	2.2	2.2	2.2	2.2
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Premix*	0.2	0.2	0.2	0.2	0.2	0.2
Toxin binder	0.1	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100	100
Analyzed chemical composition						
Energy (Kcal/kg)	2916	20932	2903	2923	2893	2913
CP %	20.50	20.01	20.49	20.00	20.50	20.00

Key

* Vitamins/kg of diet: Vitamin A, 6500 IU; Vitamin D3, 3000 IU; Choline equivalents, 800 mg; Riboflavin, 5.5 mg; Pantothenic acid, 14.0 mg; Vitamin B_{12}, 0.013 mg; Folic acid, 1.0 mg; Biotin 0.20 mg; Niacin, 40.0 mg; Vitamin k, 2.0 mg; Vitamin E, 30.0 mg; Thiamine, 4.0 mg; Pyridoxine, 4.0 mg; Manganese, 70mg; Iron, 80 mg; Copper, 10 mg; Zinc, 80 mg; Selenium, 0.30 mg; Iodine, 0.40 mg.

6.4.2 Effect of substituting soybean protein with red haricot bean protein on broiler performance

Diets containing 15 and 20% red haricot beans (representing 40% and 50% SBM protein substitution levels respectively) supported a broiler performance similar to maize–soybean control diet. Diets that contained Red Haricot beans at levels above 20% (50% SBM protein replacement level) had a performance significantly poorer performance ($p < 0.05$) than control diet. There was a general decline in all performance parameters when the proportion of beans in the diets exceeded 20%. Feed intake and feed conversion ratio were not significantly different in all the diets.

Table 6.2 Performance of broilers fed diets containing increasing levels of red haricot beans

Parameters	Diets						SEM
SBM replacement level (%)	0	40	50	60	70	80	
% Red haricot	0	15	20	25	30	35	
Weight gain (g/bird/wk)	188.5 ^{a}	165.3^{ab}	161.5^{abc}	152.5^{bc}	135.8^{bc}	133.0^{c}	62.68
Feed intake (g/bird/wk)	610.3	612.8	575.3	598.5	536.3	517.5	59.04
Feed conversion ratio	3.24	3.71	3.56	3.93	3.95	3.89	0.15
Pancreas weight (%)	0.29^{a}	0.29^{a}	0.37^{ab}	0.38^{ab}	0.42^{b}	0.32^{ab}	0.05

^{abc} Treatment means with different superscripts are significantly different ($P < .05$).

A regression analysis (quadratic fit) of weight gain against level of beans produced almost a linear decline in weight with increase in percentage beans (Figure 6.1). This indicated that weight gain declined steadily with increasing levels of red haricot although this became significant at levels of beans above 20%. Pancreas weight, expressed as a percentage of body weight, was similar for all diets except for the diet which had 30% red haricot beans (figure 6.2). The broilers fed this diet had a significantly bigger pancreas ($P < 0.05$) than the other diets. The values obtained for diet with 35% beans (80% SBM replacement with beans) were also surprising since from the other diets the general tendency was for an increase in pancreas weight with increasing levels of red haricot beans.

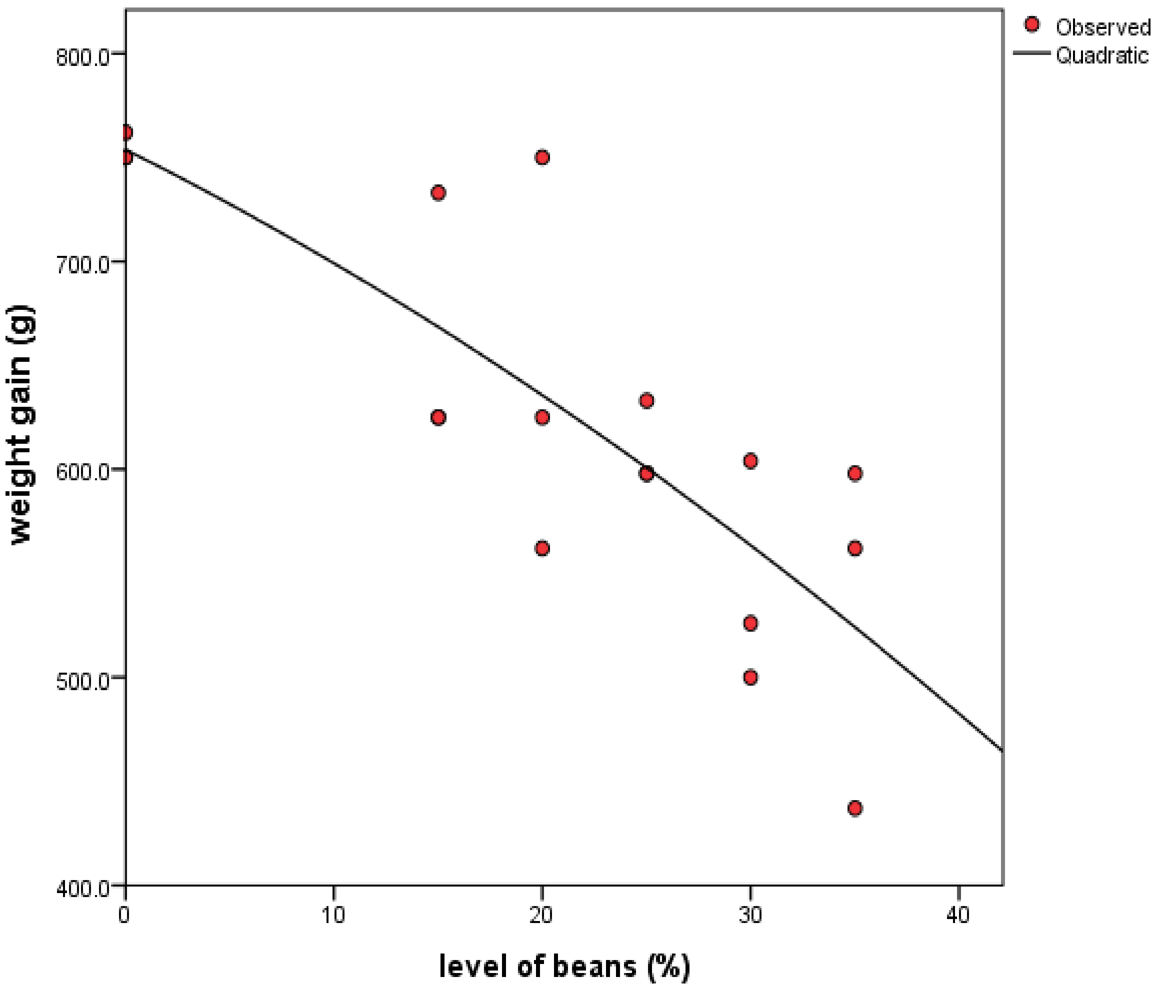

Figure 6.1 Regression of weight gain against level of beans in the diet

Y = a + b1x + b2 x^2; y = weight gain; a = 753.726; b1 = -5.025; b2 = -0.044; x = level of beans

v (correlation coefficient) = 0.82; R square (Coefficient of determination) = 0.669; P = 0.000.

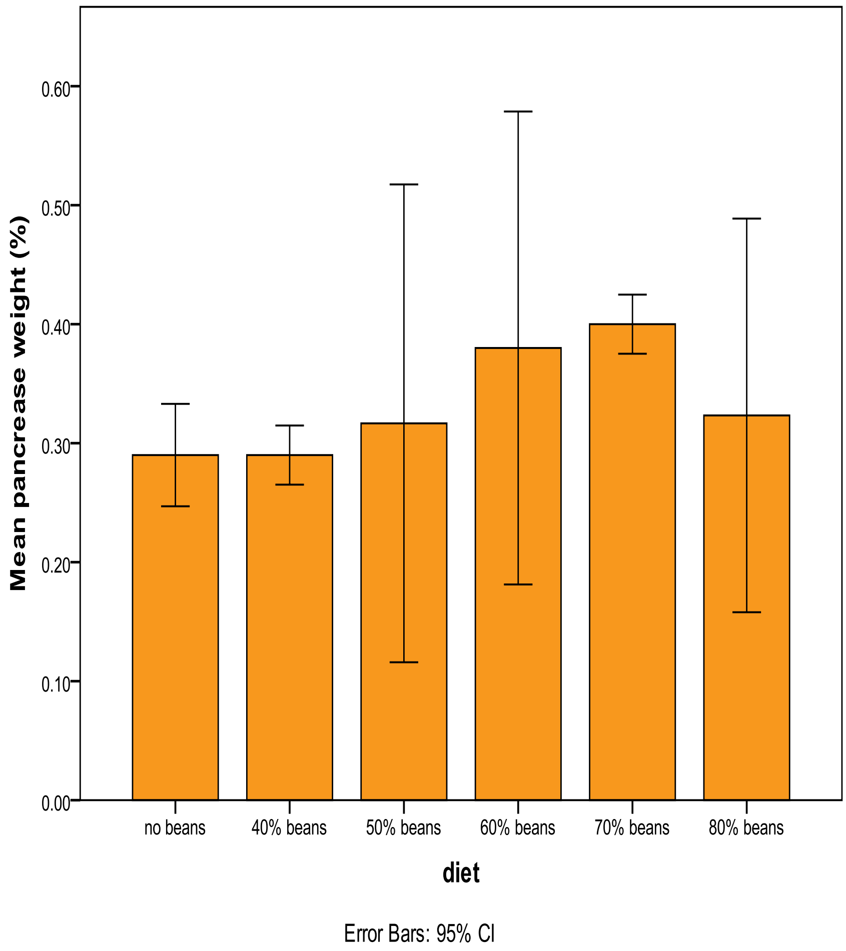

Figure 6.2 Effect of diet on broiler pancreas size

6.5. Discussion

Results obtained here indicate that beans can be included in broiler diets up to the level of 20% (50% replacement of SBM). Inclusion levels higher than 20% negatively affected growth (table 6.2, Figure 6.1). These results agree with Ofongo and Ologhobo (2007) who reported that processed kidney beans (boiled in water at 95^{0}C for 30 minutes) can be used for 50% protein for protein replacement of soybean meal or ground nut cake in broiler diets. Emiola et al. (2007a) reported good broiler performance on approximately 25% inclusion of aqueous heated and dry heated kidney beans in broiler diets. The diets did not hinder growth or organ development when compared to the control (0% bean inclusion) diet, and they had significantly better performance than the raw and raw-dehulled rations. The authors reported that birds performed slightly better on the aqueous heated diets than the dry heated versions. Feed intake (FI) and feed conversion ratio (FCR) values for control and test diets were not significantly different ($p>0.05$) from control although both showed a decreasing trend with increasing proportion of red haricot beans. Increasing levels of red haricot beans in the test diets did not affect pancreas size significantly until at 30% inclusion level (70% SBM substitution level). However, there was a general tendency to increase in size with increasing levels of red haricot beans (figure 6.2). This is a clear indication of increasing pancreatic activity with increasing levels of red haricot beans.

These results can be explained through residual effects of ANFs. Various authors have reported that cooked beans still do have residual ANF activity. Emenalom and Udedibie, (1998) and Emiola et al. (2003) reported suboptimal performance when broilers are fed processed mucuna bean meals. This was attributed to varying concentrations of residual trypsin inhibitor and haemagglutinins in the meals. The authors argued that emphasis has been placed on the various ways of inactivating the anti-nutritive factors in the legume seed and the improvement of the nutritive value while little attention has been given to the evaluation of the effects of intake of residual antinutritive factors in processed legume seeds on performance characteristics, weights, and histology of internal organs of broiler chickens. In the present results, growth is negatively affected above 20% bean inclusion level, while pancreas size is affected at 30% inclusion and feed intake not affected even at 35% level.

Residual anti-nutritive activity as evidenced by the observed tendency of pancreas size to increase with increasing levels of red haricot beans in the diet can also result in amino acid imbalances. Increased pancreatic activity has the consequence of an endogenous loss of essential amino acids that are being secreted by a hyperactive pancreas. This results in increased endogenous losses of essential amino acids, especially methionine, which is an important component of trypsin. Amino acids are diverted from the synthesis of body protein to the synthesis of lost enzymes. This loss in sulphur-containing amino acids exacerbates an already critical situation with respect to legume seeds, which are inherently deficient in these amino acids**.** In conclusion, red haricot beans cooked in boiling water for 30 minutes can be used at the level of 20% in broiler starter diets without any adverse effects on broiler performance or the size of pancreas.

CHAPTER SEVEN
7. GENERAL DISCUSSION

7.1 Introduction

Recent developments in the livestock feed industry concerning traditional protein sources such as meat and bone meal and soybean cake have triggered research into alternative protein sources for nonruminant diets. Research on alternative protein sources has been fuelled by the European Union ban on the inclusion of meat and bone meal in diets of agricultural livestock, concerns over use of genetically modified soybeans in livestock diets in addition to the high cost of importing soybean cake by the nonproducing countries. Other legumes commonly used in human nutrition but not included in livestock diets are some of the vegetable-based protein sources that are being considered (Diaz et al., 2006).

Feed cost is the major contributing factor in poultry cost of production at 60-70% of total cost of production. In Kenya the cost factor is increased by the fact that most of the feed items such as SBM, Fishmeal, vitamin and mineral premixes are imported. Given the high cost of imported feedstuffs, alternative protein sources need to be investigated to reduce cost of production. For resource poor rural farmers in Kenya, many commercially available alternatives to SBM may not reach them due to constraints such as infrastructure, supply chain issues or even politics. However, surpluses of home grown pulses such as common beans, pigeon peas, groundnuts etc might provide cheaper alternative protein options for these farmers. An important aim of research in animal production is to enhance cost effective livestock production while providing adequate animal protein and livestock by-products for human consumption. The ever increasing human population also necessitates the need to source for alternative feeds and feedstuffs for animals. It is therefore worthwhile to evaluate the potential of the many legumes grown in Kenya as poultry feeds. The primary concern with these vegetable based protein sources is related to their content of non-starch-polysacharides (NSP) and the antinutritive factors (protease inhibitors, lectins, phenolic compounds, saponins, etc.) The alfa-galactoside linkages in these polysaccharides are not broken down for digestion in the gut of monogastric animals (Kocher et al., 2000).

The negative effects of the ANF’s contained by these protein sources can however be minimized by several procedures. Many of the procedures performed to reduce the effects of ANF’s found in these legume grains have however been studied with respect to human nutrition and may also not be feasible in underdeveloped areas, or for small local feed mills or farmers mixing their own rations (Van der Poel,, 1990). Access to specialized equipment can be a limiting factor for the utilization of much of the current information available on further processing of pulses for monogastric nutrition. The heat used during the extruding and pelletizing processes has proven useful in degrading certain anti-nutritive constituents in feed ingredients, but not helpful to those who do not have access to the required equipment.

In consideration of this deficit, the feed processing techniques used in these experiments were developed to mirror conditions that can be found at village level where producers cannot depend on the environmental consistency provided by insulated buildings and thermo-regulated ventilation. These limitations are the basis for the procedures used throughout the feed processing and environmental design phases of the experiments performed in this work. Enzyme addition was chosen because it is one of the most utilized and most studied processes of eliminating ANFs and/or improving diet utilization (Cowieson et al., 2003). Other effective methods used include feed processing procedures such as cooking, soaking, and fermentation.

The objectives of this study were therefore to; i) Document the nutritional and ant-nutritional composition of common bean cultivars grown in Kenya, ii) Evaluate the effects of processing on common bean ANFs and utilization of common beans by broilers, iii) Evaluate the effects of partial or complete substitution of soybean meal with raw or processed common beans on broiler performance and iv) Evaluate the effects of enzymes and probiotics on common bean ANFs and utilization of diets based on raw or processed common beans.

7.2 Composition of common bean varieties grown in Kenya

Common beans grown in Kenya are high in anti-nutritive factors. Due to the presence of these factors , some form of processing is necessary before they can be utilized in animal feeds. Processing through heat treatment and fermentation can be used to reduce the content of the anti-nutritive factors in common beans. Fermentation marginally reduced the content of trypsin inhibitor in red haricot bean but caused substantial reduction in haemagglutinins. On the other hand heating beans for 30 minutes in boiling water inactivated trypsin inhibitor and haemagglutinins. This is in agreement with results of Emiola et al. (2007).

7.3 Effect of processing on common bean ANFs and utilization by broilers

7.3.1 Cooking

In general, the results showed that soaked and boiled red haricot beans can be included in broiler starter diets at the level of 20% without affecting broiler performance. In chapter three, cooking red haricot beans for 30 minutes, reduces ANFs to safe levels. These findings are consistent with published data reported by earlier researchers (Farrell et al., 1999; Bennet, 2002). Farrell et al. (1999) reported that feeding 200 g/kg faba bean to broilers grown to 21 days of age gave good growth response and feed efficiency. In their study, the weight gain and feed per gain were found to improve with increasing inclusion levels of faba beans.

The use of appropriate processing temperatures and heating method is critical for the elimination of heat-labile ANFs found in legume seeds (D'Mello, 1991; Thorpe and Beal, 2001). In chapter four it was reported that oven heating red haricot beans at 100^0C for 20 minutes had very little effect on ANFs and birds fed diets based on those beans performed similar to birds fed raw beans. Different heating levels have also been reported to leave varying levels of residue TIA and lectins (Farhoomand and Poure, 2006; Osman, 2007; Devaraj and Manjunath, 1995). Under- processing therefore will fail to deliver full benefits on the digestibility of amino acids, since the ANFs will not be fully eliminated.

Excessive heat treatment, or over processing, on the other hand, will also lower amino acid digestibility since amino acids may be destroyed or become unavailable due to the formation of indigestible complexes. Herkelman et al. (1991) in experiment to determine effect of heating time on anti-nutritive factors in full fat soybeans reported that chicks fed full-fat soybeans achieved maximum performance when the soybeans were heated at 121^0C for 40 minutes, further heating for 60 or 90 min reduced performance. The authors also noted that trypsin inhibitor activity remained constant with heating beyond 40 minutes. In the current study it has been shown that cooking beans in boiling water for 30 minutes is enough to reduce ANF to safe levels. Increasing temperatures may also change the relative composition of secondary structures and alter the aggregation behaviour. Temperatures up to 70^0C usually affect most proteins reversibly or partially, while temperatures between 70 to 100^0C will break hydrogen bonds, disulphide bonds and the alpha helix secondary structure. Heating between 100 and 150^{0}C damages tertiary protein structures. Van der Poel (1992) evaluated the effects of different extrusion conditions on the ANFs and protein digestibility of two cultivars of peas. It was shown that the reduction in the levels of trypsin inhibitors and lectins was dependent on the processing variables such as the moisture level, the temperature and heating time.

The results of this work therefore are in agreement with work published by other researchers. In the second experiment (chapter four), oven heating of pre-soaked beans for 20 minutes had little effect on performance of birds and the birds recorded very obvious signs of poisoning while feeding the same beans which were however boiled in water for 30 minutes (chapter five and six) supported broiler performance similar to control diet. Similar results were published by Pone and Fomunyam (2004) who roasted kidney beans at 100^{0}C in a 20 cm layer for 30 minutes in trays. When 20% of the diet was toasted kidney beans, experimental birds had poorer performance than birds fed soybean meal as the main protein source. Emiola et al. (2007b) also replaced 50% of the dietary soybean meal with kidney bean meal treated by one of three methods: aqueous heating (kidney beans were added to 100^{0}C water for one hour and oven dried at 85^{0}C for 48 hours), dry toasting (a thin layer of seeds were oven heated at 120^{0}C, stirring occasionally until the beans turned from white to golden- about 20-25 minutes), and dehulling. The authors reported that average daily gains and feed conversion efficiencies were higher in the aqueous heat treated beans than either dry toasting or dehulling. In the present work, cooking of the beans in the third (chapter 5) and fourth (chapter 6) experiments for 30 minutes in boiling water reduced deleterious effects significantly and broiler performance was similar to control diet.

Heat treatment destroys the activity of the trypsin inhibitors and other ANFs in common beans, the amount of destruction being a function of time and temperature (Herkelman et al., 1991). In chapter three, heating common beans for 20 minutes did not result in maximum rate of reduction in TIA and lectins. This was clearly illustrated in chapter four where using beans toasted at 100^{0}C for 20 minutes resulted in poor performance of birds and large pancreas size compared to the control. However, in chapter five and six, using beans boiled for 30 minutes at 100^{0}C, maximum rate and efficiency of weight gain and a maximum decrease in pancreas weight of chicks was achieved at 50% substitution level. Under the heating conditions used in this research therefore it observed that heating common beans for 30 minutes at 100^0C was required to lower the concentration of the trypsin inhibitors and other antinutritive compounds to permit efficient utilization. Excessive heat or heating time reduces the availability of amino acids due to the Maillard reaction (Del Valle, 1981) and tends to destroy certain amino acids **(**Chae et al., 1984).

7.3.2. Fermentation

In chapter three of this work it was reported that solid state fermentation with EM reduced lectin content by 94% while TIA reduced by 22%. However, broilers fed diets containing fermented red haricot beans performed as poorly as broilers fed raw red haricot beans. Solid state fermentation without precooking may not allow adequate contact between the microbial enzymes and the substrate for effective hydrolysis of ANFs to occur (Thorpe and Beal, 2001). This may explain the poor performance of broilers in this study fed diets based on either partial or complete substitution of SBM protein with fermented bean protein.

These results however support the view that the incorporation of fermentation processes into other simple food processing technologies may offer good prospects for detoxification of food sources while simultaneously improving flavour, texture and colour of the raw material. A combination of cooking and fermentation may produce better results than either used alone.

7.4 Effect of feed additives on red haricot anti-nutritive factors and utilization by broilers

7.4.1 Effect of enzymes

During the past two decades, the use of exogenous enzymes has become a common practice in the feed industry due to their effectiveness and lower costs. According to Sheppy (2001) and McCleary (2001), the main objectives of enzyme supplementation in poultry diets are to (i) destroy or lower the content of anti-nutritional factors; (ii) increase the availability of nutrient components such as starch and proteins that are either enclosed within fibre-rich cell walls and, therefore, not as accessible to endogenous digestive enzymes; (iii) breakdown specific chemical bonds in raw materials which are not usually broken down by the animal's own enzymes; (iv) supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate; (v) reduce the variability in nutritive value between samples of a feedstuff, (vi) improve gut health and (vii) decrease nutrient overload in the manure.

Five main types of enzymes are commonly used in poultry diets, which are NSP-degrading enzymes (i.e. xylanase and β-glucanase), protein-degrading enzymes (protease), starch-degrading enzymes (i.e. amylase), phytic acid-degrading enzymes (phytase) and lipid-degrading enzymes (lipase) (Sheppy, 2001; Mcleary, 2001). It is important to note that feed ingredients typically contain more than one anti-nutritive factor and, as a result, the addition of multienzymes may be more effective to improve nutrient digestibility. The current experiment used a multienzyme, Allzyme SSF from Alltech International which contained amylase, cellulose, phytase, xylanase, Beta-glucanase, pectinase and protease activities. In this study the diets with enzymes however did not perform better than diets without. Similar trends were reported in the experiments reported in chapter four and five. The results however indicated that enzymes had effects on fibre and denatured proteinous ANFs but little or no effect on raw proteinous ANF's. Using a similar multienzyme as used in this work, Endofeed W enzyme, obtained from *Aspergillus niger*, Hajat (2010) reported a significant reduction in weight gain and feed intake by birds fed Maize- wheat-SBM diets supplemented with the multienzyme at 500 g/ton.

Exogenous NSP enzymes are routinely used to mitigate the adverse effects of NSP and to minimise the variation in AME and also the performance of poultry fed diets based on viscous grains. The proposed mechanisms by which these enzymes improve energy and nutrient utilisation include degradation of NSP in the cell wall matrix and the release of encapsulated nutrients, lowering of digesta viscosity in the intestinal tract, increased accessibility of nutrients to endogenous digestive enzymes, stimulation of intestinal motility and improved feed passage rate. Legume NSP are more complex in structure than those in cereals, containing a mixture of colloidal polysaccharides called pectic substances (galactouronans, galactan and arabinans). Neutral polysaccharides such as xyloglucans and alactomannans have also been reported. The practical substrates generally targeted in practical corn-based diets are phytate, arabinoxylans, Beta-glucans and starch. Cellulose is not a practical target in poultry as no enzyme system currently exists that would efficiently and cost-effectively fully release glucose from this NSP within the constraints of the bird's gastrointestinal tract. A total depolymerisation of the complex NSP of grain legumes therefore requires extremely complex enzyme activities (Choct, 2006). An effective enzyme to degrade pectic polysaccharides present in commonly used vegetable protein sources for poultry is therefore yet to be developed.

The high excreta wetness in birds fed diets containing red beans may therefore be, in part, due to the high NSP content in the red bean seeds. Wet droppings may increase the production of gases (that is, ammonia and hydrogen sulphide) and fly and rodent populations in pig and poultry sheds. These can affect the well-being of the animals by increasing stress and lowering air quality, and they can affect the health of the staff who work in the units (Donham, 1995).

Though the exogenous NSP enzymes may have a potential role to play, their addition in this experiment did not reduce wetness. This supports the view that the effect of different glycanases in monogastric animals depend on the site of the breakdown of the NSPs in the gut and the molecular sizes of the released products which differ greatly. The degree of and type of benefit of NSP enzymes also depend on the nature of the NSP present, i.e. soluble (Mathlouthi et al., 2002) or insoluble (Jaroni et al., 1999). Viscous fibre prolongs gastric emptying and slows transit time (Malkki, 2001), which in growing animals alters nutrient digestibility and generates performance losses. Insoluble fibre/NSPs can affect gut transit time, gut motility and may also hinder the ability of endogenous enzymes to gain access to their respective substrates (Choct, 2001). Insoluble fibre/NSPs do not cause viscosity but these cell-wall components can encapsulate nutrients inside intact cell walls. These important differences between soluble and insoluble fibre determine the efficacy of an enzyme in reducing excreta moisture. Legume NSP are also much more complex in structure than those present in cereals and, therefore, the use of "classical" NSP-degrading enzyme products tends to provide limited and inconsistent responses (Broz and Ward, 2007). Over depolymerization of NSPs may yield high amounts of osmotically active oligomers in the gut, which in turn increase the viscosity of digesta and moisture content of the excreta (Choct and Annison, 1992).

The tendency to reduce weight gain by birds fed cooked common beans (chapter five) on addition of enzymes reported in this experiment may therefore be due to increased content of soluble NSP resulting to increased viscosity and gut fermentation. Elevated levels of intact soluble NSPs have been reported to detrimentally increase the activity of fermentative microorganisms in the small intestine (Choct et al., 1996). Excessive fermentation in the small intestine may interfere with the normal physiological process of nutrient digestion. As often noted, adding antibiotics to poultry diets that have highly soluble NSPs markedly improved bird performance (Misir and Marquardt, 1978). Cooking reduces soluble NSP but has no effect on insoluble NSP. Partial breakdown by enzymes may have led to increased solubility and viscosity and a significant increase in pancreas weight and reduction in performance of birds fed diets with cooked beans and enzymes.

Fermentation on the other hand has a marked effect on both soluble and insoluble fibre. This might explain the different outcomes produced by enzymes in diets with fermented and cooked red beans. In chapter five, enzymes addition to diets with boiled beans tended to reduce weight gain, feed intake and FCE while increasing pancreas weight and faecal wetness in broilers. On the other hand enzymes had no effect on performance and pancreas weight of birds on diets with fermented beans. Wetness was 10% higher for cooked beans-enzyme diet than fermented beans-enzyme diet. It is therefore possible that partial NSP hydrolysis by the multenzyme led to increased levels of soluble fibre in digesta with consequent poor nutrient utilization. Partial hydrolysis may also have liberated residual TIA leading to the cooked bean diets resulting in a larger pancreas while no pancreas effect was observed in diets with fermented beans.

7.4.2 Effect of probiotics

In this study (Chapter five) addition of probiotics to the diet with cooked red haricot beans (T3) tended to reduce weight gain and feed intake while increasing FCR of broilers compared to birds on cooked beans without probiotic. Botlhoko (2009) using EM in feed (50g/kg) and water (50mls/l) reported reduced water intake and poor performance in broilers. Pandey (2001) also reported no positive effects on broiler performance when chicks were provided with drinking water containing 5mls/l EM, while G-probiotic resulted in significant gains. Anjum et al. (2006), showed great increases in body weight gain among broilers after five weeks of Probiotics introduced into the animals feed in three different ways: 1ml/L EM in drinking water, 30g/Kg EM in feed, both 1mL/L in drinking water and 30g/Kg in feed.

The significance of gut micro flora to the nutrition of chickens is not well documented. Probiotics are expected to provide both nutritional and protection benefits to broilers through fermentation of products and prevention of colonization by pathogens. Lactobacillus species are the most important probiotic strain and produce large amounts of lactic acid from monosaccharides and disaccharide substrate leading to a lower *PH* which is fatal to many bacteria (Maritz, 2005a). However, excessive fermentation in the small intestine may interfere with the normal physiological process of nutrient digestion. As often noted, adding antibiotics to poultry diets that have highly soluble NSPs markedly improved bird performance (Misir and Marquardt, 1978). Elevated levels of intact soluble NSPs detrimentally increased the activity of fermentative microorganisms in the small intestine (Choct et al., 1996). A study by Safaloah and Smith, (1999) tested the effects of probiotics (EM) as an alternative to antibiotics (Zinc Bacitracin); broilers given 30g/kg of probiotics combined with zinc Bacitracin had the greatest weight gain when compared to control or those given probiotics, or antibiotics alone. Broilers given probiotics alone were also significantly different from the control. At 42 days of age, the control broilers had gained 2065.99 grams of weight compared to those with probiotics in their feed at 30g/kg and no AB with a gain of 2091.70. This is in contrast with current results where EM tended to reduce gain compared to control. Addition of enzymes such as xylanase which largely eliminates fermentation in the small intestine has been shown to improve the performance of birds. A sudden change in the gut ecology (from an aerobic or facultative anaerobic environment to a strictly anaerobic one) may induce gastrointestinal stress and severely affect the normal physiological processes. EM consists of a diverse group of naturally occurring, non-pathogenic, facultative anaerobic and aerobic microorganisms, which include high populations of lactic acid bacteria (*Lactobacillus* and *Pedicoccus)* and fewer amounts of photosynthetic bacteria, yeast, *bacillus sp.,* actinomyces and other organisms.

EM works by dominating the microbial ecology with organisms that exploit a fermentative pathway and therefore creates low *(pH)* by production of lactic acid and volatile fatty acids (Yongzhen and Weijiong, 1994). High content of complex NSP found in beans may explain why EM tended to reduce performance of diets with beans. The weight of pancreas tended to increase on addition of EM to diet with cooked beans. This indicates increased toxicity with addition of probiotics. This indicates an increase enzyme activity and digestion as suggested by Fuller (2001). Increased toxicity could be due to residual TIA released by microbial activity. Production performances due to EM has been reported to be a function of improving feed bioavailability, balancing gastrointestinal microorganisms, and enhancing immunity when probiotic strains are ingested (Safaloah and Smith, 1999). Variations in the effects of probiotics on broilers from various studies obtained may be due to the differences in the strains and forms of bacteria used and in the concentration of the dietary supplementation (Jin et al., 1997b). Diets with high CF may diminish the gains due to excessive fermentation in the gut. Excessive fermentation may be reduced by use of EM together with antibiotics, enzymes or diets with low fibre. EM should be added to drinking water for broilers at a dilution ranging from 1:1000 up to 1:10,000 and can be made available to the animals continually or periodically throughout the growth cycle (Yongzhen and Weijong, 1 994).

CHAPTER EIGHT

8. CONCLUSION AND RECOMMEDATIONS

8.1 Conclusion

The following conclusion can be drawn from the present research:

Experiment one:

1. Common beans grown in Kenya have a nutritive and anti-nutritive composition comparable to beans grown else where (did you analyze such beans to reach this conclusion?)
2. Soaking red haricot beans for 24 hours and cooking in boiling water for 30 minutes is effective in reducing anti-nutritive factors to safe levels
3. Solid state fermentation is only partially effective in eliminating red haricot bean ANFs

Experiment two:

1. Soaking red haricot beans for 24 hours followed by oven heating for 20 minutes has no effect on anti-nutritive factors or bean utilization.
2. Enzyme supplementation had minimal effect on common bean ANFs or utilization

Experiment three:

1. Red haricot beans soaked for 24 hrs, cooked in boiling water for 30 minutes and used at the rate of 20% in broiler starter diets can support a boiler performance equal to maize–SBM control diet
2. Enzymes and probiotics have no beneficial effect on utilization of diets containing 20% raw, cooked or fermented red haricot beans

Experiment four

1. Red haricot beans soaked for 24 hours and cooked in boiling water for 30 minutes can be included in broiler starter rations up to the level of 20% without affecting broiler performance.

2. Inclusion levels of processed red haricot beans at levels higher than 20% negatively affects broiler performance. From this work it can therefore be concluded that antinutritive factors in local red haricot beans are eliminated by soaking for 24 hours and cooking in boiling water for 30 minutes and that these processed beans can be included in broiler rations up to the level of 20%.

8.2 Recommendations

It is recommended that research be conducted on:

1. Effects of combined utilization of fermentation and cooking as processing methods
2. Effects of liquid state fermentation vs solid state fermentation of beans.
3. Evaluate the effects of first treating common beans with enzymes before mixing them with other feed ingredients to reduce dilution effects and improve enzyme – substrate interaction.

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