NUTRIENT ENRICHMENT OF PINEAPPLE WASTE THROUGH FUNGAL FERMENTATION FOR UTILIZATION AS POULTRY FEED

BY

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I56/5743/2003

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (BIOTECHNOLOGY) OF KENYATTA UNIVERSITY

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Nutrient enrichment of pineapple waste
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

I dedicate this work to my parents, the late Eric Omwango and Mary Omwango, whose support gave me a chance in life.
ACKNOWLEDGEMENT

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<tr>
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<td>ANOVA</td>
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<td>A. Niger</td>
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<td>FPW</td>
<td>Fermented pineapple waste</td>
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<td>IB</td>
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<td>LSF</td>
<td>Liquid state fermentation</td>
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<td>M</td>
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<td>MKU</td>
<td>Mt. Kenya University</td>
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<td>ml</td>
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<td>NFE</td>
<td>Nitrogen free extract</td>
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<td>nm</td>
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ABSTRACT
The use of substitutes for commercial feeds such as crop residues and agro-industrial wastes has been proposed as a sustainable means of livestock production. However, the research carried out in the recent past shows that problems of low crude protein, mineral and vitamin content; reduction in availability of certain mineral elements; high ash, high crude fiber content and poor digestibility limit their use. The enrichment by microbial fermentation to alleviate these problems has been proposed but the nutritional value of the subsequent feed for animal consumption has not been fully elucidated. This study investigated whether fermentation with various methods of pineapple waste (PW) at varying times using the fungi Aspergillus niger and Trichoderma viride could improve its nutrient content. In addition, the study investigated the conversion efficiency of the nutrient enhanced PW as feeds in poultry. Five test diets were formulated using PW that recorded significant nutrient enrichment after fermentation. Diet A, formulated using PW fermented by solid state fermentation (SSF) using A. niger for 72 hours mixed in the ratio of 1:1 with a standard commercial broiler feed (SCBF). Diet B, formulated using PW fermented by SSF using T. viride for 96 hours mixed in the ratio of 1:1 with SCBF. Diet C, composed of SCBF representing the control diet. Diets D and E, composed of PW fermented by SSF using A. niger for 72 hours and T. viride for 96 hours, respectively.

Results show that fermentation of PW by SSF using the fungi A. niger and T. viride significantly (P < 0.05) enriches the nutrient content of the waste, particularly increasing the crude protein and ash content while lowering the crude fiber content. The most significant nutrient enrichment was recorded at 72 hours of fermentation by SSF using A. niger and at 96 hours of fermentation by SSF using T. viride. In liquid state fermentation (LSF) method, the nutrient enrichment of PW is evident though significantly (P < 0.05) lower compared to SSF. Indiscernible changes were noted in the mineral content of PW under both fermentation methods. Dry matter increased significantly (P < 0.05) as fermentation progressed with the highest values recorded at 96 hours. The study established no significant differences (P > 0.05) in the fermentation abilities of the two fungi, A. niger and T. viride. SSF was the most viable fermentation method than LSF and fermentation periods, 72 and 96 hour found to be equally viable. Results of feeding trials of the test diets showed that broilers fed on diets A and B had significantly (P > 0.05) lower mean body weight and feed intake than those fed on diet C, while those fed on diets D and E died within the first week of the feeding trials. As such, the level of inclusion of fermented pineapple waste (FPW) in the test diets in this study had a negative effect on the performance of the broilers. Despite this, FPW may be a potential supplement in feed formulation due to its non toxicity and non pathogenicity.
CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The livestock sector has emerged as a key economic component in various third world countries. For example, market-oriented small-scale dairy enterprise has the potential to offer important income and employment opportunities for resource-poor producer households and for rural and urban poor through their participation in processing and marketing (Staal, 1996; Okantah, 1995; MOAC/SUA/ILRI, 1998). One of the constraints limiting smallholder livestock production especially in the third world countries is inadequate livestock feeds (Tequia and Beynen, 2005). The high level of competition between man and livestock for available feed ingredients such as maize has posed a great concern to nutritionists over the years particularly in developing countries (Fasuyi, 2005).

The fact that feed alone accounts for up to 70 – 80% of the recurrent production inputs in intensive monogastric animal production also makes the sourcing for alternative feed ingredients expedient (Fasuyi, 2005). The conventional commercial feeds are becoming expensive to afford, therefore substitutes for conventional feeds such as crop residues and agro-industrial wastes have been proposed as a sustainable means of livestock production (Akinfala, Aderibigbe and Matanmi, 2002; Maphosa et al., 2003; Iyayi and Aderolu, 2004; Fasuyi, 2005; Iyayi and Fayoyin, 2005; Tequia and Beynen, 2005; Ukachukwu, 2005).
Many developing countries including Kenya have agricultural based economies and as such produce tons of agricultural products annually. A large portion of these vast amounts is used for production of agro-industrial products, thus production of tons of agro-industrial waste. Some of these wastes are left unutilized often causing environmental pollution and hazards. Alternatively, these wastes are recommended as tremendous sources of organic raw materials and are potentially available for conversion into useful products such as animal feeds (Iyayi and Fayoyin, 2005; Ukachukwu, 2005).

Agro-industrial wastes in recent times have been the focus of research in animal nutrition especially for monogastric animals (Iyayi and Aderolu, 2004; Iyayi and Fayoyin, 2005; Ukachukwu, 2005; Emiola et al., 2006; Iyeghe-Erakpotobor, Osuoh and Olugbemi, 2006). In fact, many feeds that can be fed alternatively at cheaper cost to monogastric livestock are based on the use of agro-industrial waste that are of no food value to humans (Iyayi and Fayoyin, 2005). To further develop sustainable livestock production in Kenya, it is important to explore the possibilities of utilizing locally available agro-industrial wastes from food trade and processing industries.

However, numerous research findings have indicated that extensive use of agro-industrial waste as animal feeds has encountered some nutritional and disease problems, including low crude protein, mineral and vitamin content; reduction in availability of certain mineral elements; high ash and crude fiber content causing poor digestibility (Devendra, 1988; Lattimore and Fine, 1982). The use of microorganisms to overcome these problems and improve nutritional value of agro-industrial wastes, thereby offering the potential to
make dramatic contributions to sustainable livestock production has been well documented (Iyayi and Aderolu, 2004; Fasuyi, 2005; Iyayi and Fayoyi, 2005; Tequia and Beynen, 2005; Ukachukwu, 2005).

Microorganisms as a source of protein is one solution to the problems stated. The single cell of a microorganism is a perfect protein factory under controlled conditions in a fermentor, the culture of single cells can effect a highly efficient transformation of simple substances into protein and other important nutrients (Saquido, Cayabyab and Flordeliz, 1975). One such microorganism is fungus. The utilization of fungi for nutrient enhancement in agro-industrial waste has been studied for years and their efficiency shown in substrates such as lignin, cellulose and hemicellulose polymers found in agro-industrial waste (Howard et al., 2003).

However, the application of this biological strategy has not been evaluated in Kenya and may be an exploitable avenue for the improvement of agro-industrial wastes as a means of increasing livestock productivity and enhanced environmental sustainability. In recognition of this potential, a better understanding of this technology is required. This study was therefore conducted to investigate the potential use of fungi; Aspergillus niger 2228 (A. niger) and Trichoderma viride 2596 (T. viride) for nutrient enrichment of PW as animal feed supplement and to evaluate conversion efficiency of such upgraded wastes in poultry. It is hoped that the results shall provide more knowledge on the application of microorganisms in recovering agro-industrial waste for use as feed supplement thus enhancing livestock production and environmental sustainability.
1.2 Literature review

1.2.1 Livestock production

1.2.1.1 The feed challenge

The livestock sector has emerged a key economic component in various third world countries. The main constraints limiting smallholder livestock production especially in the third world countries is inadequate livestock feeds. The feed challenge is particularly acute for small-scale farmers. The conventional commercial feeds are becoming expensive to afford. This is occasioned by the high cost of the feed ingredients used in feed formulation that contributes to the high cost of the finished feed. This is not only an inflationary factor but also because of the reducing availability of feed ingredients (Ahmad, Miah and Hossain, 2006).

Most developing countries have problems with the supply and quality of feed and very limited numbers of feed ingredients are available to choose from for the formulation of balanced diets. This is as a result of the constant competition amongst human and animals for the same ingredients (Hossain, Ahammad and Howlider, 2003; Iyai and Aderolu, 2004; Fasuyi, 2005).

Increased concerns of the feed producers regarding the quality and cost of feed have changed the approach of using conventional feed and therefore, invoked the suggestions of unconventional or nontraditional feeds in feed formulation (Tequia and Beynen, 2005). The fact that feed alone accounts for up to 70-80 % of the recurrent production inputs in intensive monogastric animal production also makes the sourcing of alternative feed
ingredients expedient (Fasuyi, 2005). Therefore, substitutes for conventional feeds such as crop residues and agro-industrial wastes have been proposed as a sustainable means of livestock production (Akinfala, Aderibigbe and Matanmi, 2002; Maphosa et al., 2003; Iyayi and Aderibigbe, 2004; Fasuyi, 2005; Iyayi and Fayoyin, 2005; Tequia and Beynen, 2005; Ukachukwu, 2005).

1.2.1.2 Alternative feed sources

From the production, processing and consumption of agricultural products there are a great variety of remainders, which create increasing problems of elimination from the environment (Albores et al., 2006). Some of the by-products of milling, brewing, sugar and oil industries are traditionally used for livestock feeding. The use of agro-industrial waste in the development of livestock production in Africa has been reported (Tequia and Beynen, 2005). In view of the reported challenges faced by livestock farmers regarding feed costs and availability, there is a need to develop technologies for using efficiently by-products obtained from food and agro-industries and other non-conventional feeds as protein and energy supplements.

Waste recovery has been advanced as a method for preventing environmental decay and increasing food supplies (Bellamy, 1974). The potential benefits from successful recycling of agricultural wastes are enormous especially since it is the most effective method for producing animal and human food from lignocellulose material that are of little nutritive value and are therefore used as fuel (Bellamy, 1974). Around the world, agro-industries have increasingly been recovering agro-industrial waste as by-products in
an attempt to reduce pollution problems and increase income on the same product by
converting them into feed products (Iyayi and Aderolu, 2004; Fasuyi, 2005; Tequia and
Beynen, 2005; Ukachukwu, 2005).

Despite the huge potential of using agro-industrial waste as an alternative feed source in
solving the feed challenge, numerous research findings have indicated that extensive use
of agro-industrial waste as animal feeds has encountered some nutritional and disease
problems (Devendra, 1988; Lattimore and Fine, 1982). The problems include low crude
protein, mineral and vitamin content; reduction in availability of certain mineral
elements; high ash and crude fiber content causing poor digestibility (Devendra, 1988;
Lattimore and Fine, 1982). Various methods of improving on the shortcomings
encountered with the use of agro-industrial waste as feed have been proposed, including
mechanical, chemical and biological methods (Bellamy, 1974; Saquido, Cayabyab and
Flordeliz, 1975).

Among the many methods proposed, the biological method of biodegradation or
fermentation, particularly using microorganisms for single cell protein (SCP) production
seems to be the most suitable especially for tropical developing countries (Sinegani et al.,
2005). This is because it may be the only method for large-scale production that does not
require a concomitant increase in energy consumption (Bellamy, 1974). A great deal of
interest has been focused on the potential of converting agricultural wastes to SCP
(Bellamy, 1974; Oshoma and Ikcnebomeh, 2005). The impetus behind SCP production
lies partly in the need for more protein and partly in the commercial increase in the
economic advantages gained by substitution of SCP for the conventional protein used in livestock feeding (Oshoma and Ikenebomeh, 2005).

1.2.1.3 Single cell protein (SCP) production from agro-industrial waste

Biotechnology is a motor of technological advancement in both the developed and developing countries (DaSilva, Baydoun and Badran, 2002). One of the broad sectors of biotechnology has been in its application in industry and to environmental problems through biodegradation and bioremediation of waste (DaSilva, Baydoun and Badran, 2002). The advent of biotechnology, specifically fungal biotechnology, with its inexpensive mode of application has been used as a tool for the effective conversion of agro-industrial wastes into useful products (Iyayi and Aderolu, 2004).

The biotechnological method of using microorganisms through fermentation or SCP production to overcome the nutritional and disease problems encountered with the use of agro-industrial waste as feed by improving their nutritional value thereby offering the potential to make dramatic contributions to the feed adequacy challenge has been well documented (Bellamy, 1974; Senez, Raiabovlt and Deschamps, 1980; Guerra et al. 1986; Stamford et al., 1987; Duru and Uma, 2003; Iyayi and Aderolu, 2004; Sinegani et al., 2005; Albores et al., 2006; Belewu, Belewu and Bello, 2006; Oboh, 2006).

Stamford et al. (1987) concluded that cashew nut waste fermented with A. niger had chemical composition and organoleptic characteristics that made it suitable for consumption by animals. Iyayi and Aderolu (2004) concluded that fungal biodegradation
of agro-industrial waste is an effective tool for the enhancement of the nutritive value of agro-industrial wastes and the utilization of these fermented wastes; brewers dried grains, palm kernel meal and rice bran spared the use of half the quantity of maize in conventional layer chicken diet in addition to lowering the cost of egg production by up to 28 %. Albores et al. (2006) while proposing fermentation of agro-industrial waste, citrus bagasse and rice bran by *Pleurotus* spp for use as ruminant feed, reported higher protein levels, better conservation of the substrate and increased *in vitro* digestibility of the fermentation product.

Other researches have reported on the successful biodegradation/fermentation and nutrient enrichment of a variety of agro-industrial wastes. Senez, Raiabovilt and Deschamps (1980) working with cassava, potato and banana waste, showed that after 30 hours of fermentation the rate of conversion of carbohydrate to protein in the waste was 20 to 25 % corresponding to 40 to 50 % conversion into dry weight biomass. Guerra *et al.* (1986) compared the effect of inclusion and exclusion of mineral salts KCl and FeSO₄ in the culture media during PW fermentation using the fungi *Apergillus niger*, *Trichoderma viride* and *Myrothecium verrucaria*. They reported protein increment in the substrates by up to 595 %. Oshoma and Ikenebomeh (2005) were able to report increased production of crude protein from fermentation of rice bran supplemented with glucose. Oboh (2006) upgraded the protein content in cassava waste from 8.2 to 21.5 % using a mixed culture of *Saccharomyces crevisae* and *Lactobacillus* spp. The process also significantly (*P* < 0.05) lowered the antinutrients, phytate and cyanide contents in the fermented cassava peels.
Recent studies conducted to determine the efficacy of agro-industrial waste as feed supplement in monogastric animals yielded varied results (Ajayi et al., 2005; Fasuyi, 2005; Iyayi and Fayoyin, 2005; Ukachukwu, 2005; Emiola et al., 2006; Iyeghe-Erakpotobor, Osuhor and Olugbemi, 2006). Few studies conducted on monogastric animals using agro-industrial waste enhanced by fungal fermentation yielded encouraging results. Iyayi and Aderolu (2004) used T. viride to ferment brewers dried grains, rice bran, palm kernel meal and corn bran and determined their efficacy in the diet of laying hens. They were able to spare half of the maize meal of the hen diet and produced better laying performance. Fasuyi (2005), using maize-sorghum brewers dried grains, a by-product of beer production, concluded that maize-sorghum brewers dried grain can act as an energy substitute for maize at inclusion levels of about 20 % in broiler starter diets without any adverse effect on performance, carcass characteristics and muscle development in broiler chicks.

1.2.2 Fermentation technology

Fermentation is a process of chemical change caused by organisms or their products, usually producing effervescence and heat (Ensymm, 2005). Microbiologists consider fermentation as any process for the production of a product by means of mass culture of micro-organisms while biochemists consider fermentation as an energy-generating process in which organic compounds act both as electron donors and acceptors, hence fermentation is an anaerobic process where energy is produced without the participation
of oxygen or other inorganic electron acceptors. In biotechnology, the microbiological concept is widely used (Ensymb, 2005).

Fermentation enhances the nutrient content of a substrate through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein quality and fiber digestibility. It also enhances micronutrient bioavailability and aids in degrading antinutritional factors (Oboh, 2006). The fermentation process of staples serves as a means of providing a major source of nourishment for large rural populations and contributes significantly to food security by increasing the range of raw materials that can be used in the production of edible products (Oboh, 2006).

1.2.2.1 Fermentation microorganisms

The growth of microorganisms involves complex energy based processes and the rate of growth of microorganisms is dependent upon several fermentation conditions, which should provide for the energy required for various chemical reactions (Ensymb, 2005). The production of a specific compound requires very precise cultural conditions at a particular growth rate. The rate of growth of microorganisms and hence the synthesis of various chemical compounds, requires organism specific chemical compounds as the growth (nutrient) medium. The kinds and relative concentrations of the ingredients of the medium, the pH, temperature, purity of the cultured organism, influence microbial growth and hence the production of the total mass of cells or the organism being grown, and the synthesis of various compounds (Ensymb, 2005).
When a particular organism is introduced into a selected growth medium, the medium is inoculated with the particular organism. Growth of the inoculum does not occur immediately but takes a little while. This is the period of adaptation called the lag phase (Ensymm, 2005). Following the lag phase, the rate of growth of the organism steadily increases for a certain period. This period is the log phase or exponential phase (Ensymm, 2005). After a certain time of exponential phase, the rate of growth slows down due to the continuously falling concentrations of nutrients and/or a continuously increasing concentration of toxic substances. This phase is the deceleration phase (Ensymm, 2005). After the deceleration growth ceases and the culture enters a stationary phase or a steady state where the biomass remains constant except when certain accumulated chemicals in the culture lyse the cells or other microorganisms contaminate the culture or mutation of the organisms in culture, internal contamination (Ensymm, 2005).

The growth of microorganisms or other cells results in a wide range of products. Accordingly, fermentation processes aim at one or more of the following: production of cells (biomass); extraction of metabolic products such amino acids, proteins (including enzymes), vitamins and alcohol, for human and/or animal consumption or industrial use such as fertiliser production; modification of compounds through the mediation of elicitors or through biotransformation and production of recombinant products (Ensymm, 2005).

It has been known for years that numerous kinds of yeasts, fungi, and bacteria have a direct relation, favourable or unfavourable to operations such as brewing, winemaking
and cheese-making (Saquido, Cayabyab and Flordeliz, 1975). These have emerged from small-scale or family arts to the present industrial scale. One of the major modern scientific and technological advances has been in the area of harnessing the activities of microorganisms (Saquido, Cayabyab and Flordeliz, 1975). Protein of microbial origin, also known as SCP or microbial protein can be derived from a variety of microorganisms, both unicellular and multicellular (Saquido, Cayabyab and Flordeliz, 1975).

One of the applications of microorganisms is in the search for additional sources of protein (Saquido, Cayabyab and Flordeliz, 1975). These potentially important food substances are not pure proteins but are, rather, dehydrated cells consisting of mixtures of proteins, lipids, carbohydrates, nucleic acids and a variety of other non-protein nitrogenous compounds, vitamins, and inorganic compounds (Meyer, 2004). The single cell of microorganism is perfect protein factory, which under controlled conditions in a fermenter can effect a highly efficient transformation of simple substances into protein (Saquido, Cayabyab and Flordeliz, 1975). The use of land in this case is negligible and the gain in time is great, because of the fast rate of reproduction by microorganisms (Saquido, Cayabyab and Flordeliz, 1975).

Interest in microbial protein production is high because microorganisms can utilize waste materials that cause pollution problems and are sanitary hazards (Saquido, Cayabyab and Flordeliz, 1975). In the past, the use of agro-industrial wastes like cassava waste, rice bran, cane molasses, wheat offal, brewers dried grain, maize offal, palm kernel meal, citrus, PW and other starchy residues as substrates for growing microbes has been studied
To be useful substrates for production of microbial protein, agro-industrial waste must meet the following criteria: should be non-toxic, abundant, totally redeemable, non-exotic, and cheap, and able to support rapid growth and multiplication of the organisms resulting in a biomass of high quality (Dunlap, 1975).

The main reasons for using microorganisms in the recovery of agro-industrial wastes are: first, to degrade that part of the waste that is not available for absorption by animals or man when the material is fed as such. In most cases this means that the enzymes secreted in the animal or human gastro-intestinal tract cannot, or are insufficiently able to, break down the material into components that can be absorbed. This pertains to cellulosic, hemicellulosic, and ligno-cellulosic components (van Weerden, 1979). The second purpose is to upgrade the nutritional quality of the waste by increasing its protein content, or, for monogastric animals and man, raising its content of essential amino acids (van Weerden, 1979). Additionally, the growth of microorganisms is more rapid than that of the higher plants and is attractive as high-protein crops; while only one or two grain crops can be grown per year, a crop of yeasts or fungi may be harvested weekly and bacteria may be harvested daily (Bellamy, 1974).

1.2.2.1.1 Bacteria

Bacteria have, for many centuries, contributed to food supplies for man in an indirect manner. The protein supply of the ruminant is largely dependent on the bacteria and
protozoa abundantly present in the fore-stomach of the animal, which forms, in principle, a large in vivo fermentation vessel. A limited number of bacterial species have been grown specifically for food purposes (Saquido, Cayabyab and Flordeliz, 1975). These bacteria have been used extensively for SCP production on hydrocarbons (Saquido, Cayabyab and Flordeliz, 1975). Although bacteria have a slight advantage over other microorganisms as a food and feed source because of their higher growth rates and relatively higher protein content and sulphur containing amino acids, they have been objected to because of their size that makes harvesting difficult without the use of flocculants or thickeners (Saquido, Cayabyab and Flordeliz, 1975).

1.2.2.1.2 Yeast

Yeast has probably been the most commonly used organism in the production of microbial biomass since it is already accepted both in human food and in animal feed industries (Saquido, Cayabyab and Flordeliz, 1975). Yeast based processes are the farthest advanced towards commercial production since they have many convenient characteristics such as the ability to use a wide variety of substrates; susceptible to induced and genetic variation, ability to flocculate and high nutritional value (Saquido, Cayabyab and Flordeliz, 1975). However, attention has always been drawn to the fact that yeasts appear to be deficient in essential sulphur amino acids (Saquido, Cayabyab and Flordeliz, 1975).

Several species of yeast have proven very beneficial with regard to substrate fermentation or bioremediation with only few imperfect yeasts being pathogenic (Stone, 2006). The
common species that have been used over the years for preparation of fermented foods such as bread and beer include *Saccharomyces cerevisae*, *Candida utilis* and *Kluyveromyces marxianus* (Stone, 2006). Species of yeast have been used for many years as a valuable component of animal feeds, supplying proteins and certain vitamins (van Weerden, 1979). They have been fed to animals for more than a hundred years, either in the form of fermented mash produced on the farm, yeast by-products from breweries or distilleries, or commercial yeast products specifically produced for animal feeding (Stone, 2006).

In addition, some of the large-scale industrial SCP production processes developed over the past years uses yeasts that utilize hydrocarbons such as paraffin as an energy source and carbon and hydrogen for growth and synthesis of cell constituents (Stone, 2006). Ojokoh and Uzeb (2005) studied the production of *Saccharomyces cerevisae* biomass in papaya extract and concluded that the dry yeast biomass containing 35.5 % crude protein was a potential food and feed supplement when compared to the lower limits of 8 % for cattle and poultry feed. Their study was able to realize an improvement in crude protein content from 0.1 % to 35.5 % and increment of saccharide from 9.8% to 40.7 %.

*Saccharomyces cerevisae* in combination with *Lactobacillus spp* has been found to significantly increase the protein content of cassava peels while decreasing the carbohydrate content (Oboh, 2006). In addition, the biomass decreased in antinutrient (residual cyanide and phytate) content (Oboh, 2006).
1.2.2.1.3 Fungi

The fungi kingdom offers enormous biodiversity, with around 70,000 known species, and an estimated 1.5 million species in total, most of which are filamentous fungi, which differ from the yeasts not only in their more complex morphology and development, asexual and sexual structures, but also in their greater metabolic complexity (Vining, 1990). In particular, fungi are known for production of secreted enzymes and secondary metabolites, many of which have been exploited by man (Turner, 2000). Secondary metabolites are compounds produced by the fungus that are not essential to the basic metabolism of the fungus. They are generally produced following active growth, and many have an unusual chemical structure. Some metabolites are found in a range of related fungi, while others are only found in one or a few species. The restricted distribution implies a lack of general function of secondary metabolites in fungi (Vining, 1990).

Many secondary metabolites have been found to have use in industry and medicine. Six of the twenty most commonly prescribed medications are of fungal origin (Vining, 1990). Some metabolites are toxic to humans and other animals, yet others can modify the growth and metabolism of plants (Vining, 1990). Genetic analysis of secondary metabolic pathways over the past 10 years has revealed some common themes and offered new approaches to the exploitation of natural products (Turner, 2000).

Fungi have until now not been used to a large extent in animal feeding though the utilization of fungi for protein production has been studied for years (Senez, Raiabovlt
and Deschamps, 1980; Guerra et al., 1986; Nguyen and Nguyen, 1992; Oshoma and Ikenebomeh, 2005; Sinegani et al., 2005). Other studies on fungi have been on production of industrial enzymes and organic acids such as citric acid and lactic acid (Howard et al., 2003; Pothiraj, Belaji and Eyini, 2006). Fungi have the ability to provide form and texture and hence can be harvested with ease. They have low nucleic acid content and can prosper on a variety of substrates although the growth rates vary considerably with different substrates (Saquido, Cayabyab and Flordeliz, 1975). Few fungi exhibit problems of sucrose assimilation (Saquido, Cayabyab and Flordeliz, 1975).

The efficiency of fungi in fermentation has been shown in a variety of agro-industrial wastes as substrates. Guerra et al. (1986) used A. niger, T. viride and Myrothecium verrucaria on PW, Duru and Uma (2003) used Aspergillus oryzae on cocoyam cormel, Iyayi and Aderolu (2004) used T. viride on brewers dried grains, rice bran, palm kernel meal and corn bran while Oshoma and Ikenebomeh (2005) used A. niger on rice bran. Other researchers have gone ahead to compare the efficacy of different fungal species. Iyayi (2004) determined the changes in protein, sugar and cellulose of three agro-industrial wastes after fermentation with A. niger, Aspergillus flavus and Penicillum sp. Sinegani et al. (2005) compared the biodegradation capacities of a number of saprophytic fungi, Armillaria sp., Polyporus sp. and P. chrysosporium on pea, barley, wheat, rice and wood residues.

This study identified fungi as the most suitable microorganism to determine the possibilities of enhancing the nutrient content of PW due to their better biosafety record
compared to the other microorganisms. The study particularly identified *A. niger* and *T. viride* due to their success in fermenting and enhancing the nutrient content of different agro-industrial wastes and their non-toxicity record in food or feed.

*A. niger* is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. *Aspergilli* are ubiquitous in nature. They are geographically widely distributed, and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil (Greenwood, Slack and Peuthere, 2002). Their colonies appear velvet or slightly flocculent and sporulate heavily with the spores having black or brownish black appearance with the colony diameter reaching 5 – 6 cm in 2 weeks (Greenwood, Slack and Peuthere, 2002).

The primary uses of *A. niger* are for the production of enzymes and organic acids by fermentation. The history of safe use for *A. niger* comes primarily from its use in the food industry for the production of many enzymes such as amylase, amyloglucosidase, cellulases, lactase, invertase, pectinases, and acid proteases. Citric acid fermentation to produce citric acid, an organic acid using *A. niger* is carried out commercially in both surface culture and in submerged processes. *A. niger* has also been successfully used in a
number of studies to enhance the nutrient content of various agro-industrial wastes including PW (Guerra et al. 1986; Iyai, 2004; Oshoma and Ikenebomeh, 2005).

*Trichoderma* spp. are present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. They are favored by the presence of high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere competent capable of colonizing and growing on roots as they develop. In addition to colonizing roots, *Trichoderma* spp. attack, parasitize and otherwise gain nutrition from other fungi. Since *Trichoderma* spp. grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other fungi and for enhancing plant and root growth.

Most *Trichoderma* strains have no sexual stage but instead produce only asexual spores. However, for a few strains the sexual stage is known, but not among strains that have usually been considered for biocontrol purposes. The sexual stage, when found, is within the Ascomycetes in the genus *Hypocrea*. Traditional taxonomy was based upon differences in morphology, primarily of the asexual sporulation apparatus, but more molecular approaches are now being used. Consequently, the taxa recently have gone from nine to at least thirty-three species. Most strains are highly adapted to an asexual life cycle. Thus, the fungi are highly adaptable and evolve rapidly. There is great diversity in the genotype and phenotype of wild strains. The *T. viride*, colonies are pale and very fast growing forming a colony of a diameter ranging form 5 – 8 cm in 7 days. The colony
colour changes from thrifty cottony giving rise to white sporodochial tufts which turn green as conidia develop (Greenwood, Slack and Peuthere, 2002).

*Trichoderma* spp. are highly efficient producers of many extracellular enzymes. They are used commercially for production of cellulases and other enzymes that degrade complex polysaccharides. They are frequently used in the food and textile industries for these purposes. For example, cellulases from these fungi are used in "biostoning" of denim fabrics to give rise to the soft, whitened fabric--stone-washed denim. The enzymes are also used in poultry feed to increase the digestibility of hemicelluloses from barley or other crops. *T. viride* strain has been successfully used in a number of studies to enhance the nutrient content of various agro-industrial wastes including PW (Guerra *et al.*, 1986; Iyayi and Aderolu, 2004).

1.2.2.2 Fermentation methods

There are a number of methods employed today to achieve the fermentation process in various fields of research and industry. Two methods of fermentation, solid state fermentation (SSF) and liquid state fermentation (LSF) have widely been used by various researches interchangeable to improve the nutrient content of solid wastes including agro-industrial waste (Senez, Raiabovlt and Deschamps, 1980; Guerra *et al.*, 1986; Iyayi and Aderolu, 2004; Oshoma and Ikenebomeh, 2005; Albores *et al*., 2006; Oboh, 2006). Most of the recent research activities on SSF are being done in developing countries as a possible alternative for conventional LSF, which is the main process in pharmaceutical and food industries in industrialized nations (Raimbault, 1998).
1.2.2.2.1 Liquid state fermentation (LSF)

The LSF also referred to as submerged fermentation processes involves subjecting the substrate and fermenting microorganisms in submerged conditions with water being the most common media of use, always in a free state and carbon, nitrogen, phosphorus and other nutrients in suspended or dissolved state (Carlile and Watkinson, 1996). Both filamentous and non-filamentous fungi such as yeast can grow in liquid fermentation systems. In static culture, filamentous fungi form a mat of hyphae at the surface of the liquid. However, when the liquid broth is mixed, it results in submerged growth with no surface growth. Mixing induces the formation of hyphal clumps or pellets that grow radially outwards (Carlile and Watkinson, 1996, p.480). In the case of *A. niger*, growth in liquid broths only yields pellets (Carlile and Watkinson, 1996).

Some of the advantages of LSF include; heat sterilization and aseptic control, easy control of temperature, easy pH control, easy inoculation and good homogenization of the fermentation culture. Disadvantages include; consumption of high volumes of water coupled with high amounts of polluting effluents discarded, limitation of soluble oxygen hence high levels of air required, high energy consumption and high cost of technology in terms of the equipment required (Raimbault, 1998). LSF is only economically worthwhile when done on an industrial scale, using processes that require a strict control of fermentation and which take place in a sterile environment (Raimbault, 1998). The processes are well developed in industrialized countries but are not suitable for developing countries since they are technical, labour and cost intensive (http://www.fao.org/DOCREP).
1.2.2.2 Solid state fermentation (SSF)

The SSF is a simpler fermentation process that does not require modern equipment, power supply, or sterile conditions. In addition, the capital investment is low, permitting countryside operation and the use of available manual labor (Raimbault, 1998). It has been reported that the SSF is an attractive alternative process to produce fungal microbial enzymes using agro-industrial wastes due to its lower capital investment and lower operating cost (Howard et al., 2003). The SSF process is characterized by the complete or almost complete absence of free liquid. Water, which is essential for microbial activities, is present in an absorbed or in complexed form with the solid matrix and the substrate (Howard et al., 2003). These cultivation conditions are especially suitable for the growth of fungi, known to grow at relatively low water activities (Howard et al., 2003). As the microorganisms in SSF grow under conditions closer to their natural habitats, they are more capable of producing enzymes and metabolites that will not be produced or will be produced only in low yield in submerged conditions (Howard et al., 2003).

The SSF method has the following advantages. The low availability of water reduces the possibilities of contamination by bacteria and yeast which allows for aseptic conditions in some cases, there are higher levels of aeration especially adequate in those processes demanding an intensive oxidative metabolism, simple design reactor or fermenters with few spatial requirements, low energetic requirements and small volumes of polluting effluents (Perez-Guerra et al., 2004). Similarly, SSF has some disadvantages. Only microorganisms that can grow at low moisture levels can be used, usually substrates require pretreatment such as size reduction, removal of metabolic heat generated during
growth may be difficult and the solid nature of the substrate causes problems in the monitoring of the process parameters such as pH, moisture content, oxygen and biomass concentration (Perez-Guerra et al., 2004).

1.2.3 Pineapple production

1.2.3.1 Pineapple plant

The pineapple plant (*Ananas comosus*), is the leading edible member of the family Bromeliaceae which embraces about 2,000 species, mostly epiphytic and many strikingly ornamental (Morton, 1987). The pineapple plant bears a terminal multiple fruit that continues to grow after fruiting by means of one or more auxiliary buds growing into vegetative branches that mature into the fruit while still attached to the old plant (http://www.fao.org/arg). As individual fruits develop from the flowers they join together forming a cone shaped, compound, juicy, fleshy fruit to 30 cm or more in height, with the stem serving as the fibrous but fairly succulent core (Morton, 1987). The tough, outer waxy rind, made up of hexagonal units, may be dark-green, yellow, orange-yellow or reddish when the fruit is ripe. The flesh ranges from nearly white to yellow (Morton, 1987). The pineapple plant bears its first fruit about 20 months after planting and will go on to produce another fruit 10 months later (Cirio del Monte, 2002).

The pineapple plant is native to tropical America but is now cultivated in many warm countries including Kenya (http://www.fao.org/arg). It is a tropical or near tropical plant limited to low elevations between 30° north and 25° south, a temperature range of 18.33 - 45 °C is most favorable, though the plant can tolerate cool nights for short periods
(Morton, 1987). The edible portion of the pineapple fruit contains 75 - 95 % water, the fruits are low in protein but in general contain substantial carbohydrates (Morton, 1987). The latter may include various proportions of glucose, fructose, sucrose and starch according to the type of fruit and its maturity (Morton, 1987).

1.2.3.2 Pineapple processing

The pineapple plant is cultivated predominantly for its fruit that is consumed fresh or as canned fruit and juice. World canned pineapple and pineapple juice production in 1998 was estimated at 62.5 million standard cases equivalent to 1.25 million tons (MPIM, 1999). Major world producers of canned pineapple in 1998 were Thailand (39 %), Philippines (23 %), Indonesia (14 %), Malaysia (2.2 %) and Kenya (8 %) which all together contributed to more than 80 % of total world canned pineapple production (MPIM, 1999). In Kenya, the pineapple plant is predominantly grown in Thika district of central province for commercial purposes by the Cirio Del Monte Company Kenya in a 5500 hectare plantation. Every year, Cirio Del Monte Kenya plants about 1300 hectares of land with an average of 70,000 pineapple plants per hectare, in a planned rotation. The company has a cannery that produces over 3.7 million cases and 70,000 drums of pineapple products a year (Cirio Del Monte, 2002).

During canning, the best parts are selected for sliced pineapple, others are used for tidbits, desert bits, spears, cubes, crush and beverage juices while the rest is refined into fruit syrup (Cirio Del Monte, 2002). Residual parts cores, skin and fruit ends are crushed and given a first pressing for juice to be canned as such or prepared as syrup used to fill
the cans of fruit, or is utilized in confectionery and beverages, or converted into powdered pineapple extract which has various roles in the food industry (Morton, 1987). A second pressing yields "skin juice" which can be made into vinegar or mixed with molasses for fermentation and distillation of alcohol; cleaning machete and knife blades and, with sand, for scrubbing boat decks; taken as a diuretic and to expedite labor, also as a gargle in cases of sore throat and as an antidote for seasickness (Morton, 1987).

Pineapple leaves yield a strong, white, silky fiber. Certain cultivars are grown especially for fiber production and their young fruits are removed to give the plant maximum vitality. The outer, long leaves are preferred. In the manual process, they are first decorticated by beating and rasping and stripping, and then left to rest in water to which chemicals may be added to accelerate the activity of the microorganisms that digest the unwanted tissue and separate the fibers. The rested material is washed clean, dried in the sun and combed. In mechanical processing, the same machine is used that extracts the fiber from sisal (Morton, 1987).

1.2.3.3 Pineapple waste (PW)

When the pineapple fruits are canned the crown, the outer peel and the central core are discarded as waste, called PW which accounts for about 50 % of the total pineapple fruit weight corresponding to about 10 tons of fresh pineapple or one ton of dry PW per hectare (http://www.fao.org). Cirio Del Monte Kenya produces 1500 tons of pineapple daily (Cirio Del Monte, 2002). This should translate to approximately 750 tons of PW
daily. These amounts of PW pose a major disposal problem. Presently, the Cirio Del Monte Kenya uses PW as manure or natural fertilizer in the pineapple plantations.

Waste from pineapple is low in protein content and high in fiber content specifically cellulose, hemicellulose and simple sugars (Chaiprasert, Bhumiratana and Tanticharoen, 2001). The crowns are sometimes fed to horses if not needed for planting while the final PW from the processing factories may be dehydrated as "bran" and fed to cattle, pigs and chickens (Morton, 1987). "Bran" is also made from the stumps after bromelain extraction. Expendable plants from old fields can be processed as silage for maintaining cattle when other feed is scarce. The silage is low in protein and high in fiber and is best mixed with urea, molasses and water to improve its nutritional value (Morton, 1987).

In previous research studies, PW has proved to be a good substrate for citric acid and biogas production (Chaiprasert, Bhumiratana and Tanticharoen, 2001). Guerra et al. (1986) in their study on protein enrichment of PW for animal feed were able to show that the protein content in PW could be improved by 595 % through fermentation using the fungi A. niger, T. viride and M. verrucaria.

1.2.4 Poultry (broiler) production

Poultry farming is an important small and large scale venture with the potential for rapidly increasing the availability of animal protein in the developing countries where malnutrition is a great problem (Ogbamgba and Wekhe, 2006). Total consumption of poultry meat and eggs has increased dramatically during the past five decades and
continues to increase ahead of human population growth (Hossain, Ahammad and Howlider, 2003).

Broilers refer to young chicken reared for meat production. They are fed differently from pullets which are young chicken reared for egg production. Broiler diet is predominantly composed of plant materials, mainly cereals and vegetable proteins plus little amount of animal protein (Alam et al., 2003). Broiler production has become a specialized and speedy poultry operation during the recent years all over the world. Currently the cost of feed incurs about 60 – 65% of the total cost of poultry production and protein costs about 13% of the total feed cost (Hossain, Ahammad and Howlider, 2003). Poultry production may not be remunerative if costly conventional feeds cannot be replaced by the cheaper unconventional feeds.

In Kenya, fishmeal is the common conventional animal protein source in the poultry diet formulation especially in the broiler diet. It’s a costly feed item since poultry is a competitor of human beings in respect of dry fish consumption hence when added to the diet, fish meal increases poultry production cost too much (Hossain, Ahammad and Howlider, 2003). For the above reason, it is very important to find out the possibilities of using alternative source of low cost crude protein to substitute the expensive fishmeal.

1.2.5 Feed assay techniques

Procedures that can be applied when testing the nutritional value of new components meant for inclusion in animal feeds are categorized in four tests. Analysis, acceptability,
digestibility and comparative feeding trials (van Weerden, 1979). These four different types of tests form a chronological sequence of steps in the evaluation procedure.

The first step in feed assay involves chemical analysis, a determination of the major components: protein, lipid, ash, carbohydrate, but preferably also amino acids, macro elements, and the more important minor elements. Many of the relevant analyses where possible are performed, as a complete analytical profile provided much early information about the potential nutritional value of the product to be tested. Proximate analysis is the most common method used to determine protein, fat, ash and carbohydrate content in the component meant for inclusion in animal feeds (AOAC, 1998).

Specific minerals may be analyzed by atomic absorption spectroscopy (AAS). The mixture to be analyzed is ignited in a flame of temperature ranging from 2100°C to 2800°C depending on the fuel gas used. During combustion, atoms of the element of interest in the sample are reduced to the atomic state. A light beam from a lamp whose cathode is made of the element being determined is passed through the flame into a monochromometer and detector. Free, unexcited ground state atoms of the element absorb light at characteristic wavelengths. This reduction of the light energy at the analytical wavelength is a measure of the amount of the element in the sample (Okalebo, Gathua and Woomer, 1993).

The second step in feed assay is acceptability trials. These acceptability studies conducted through feeding trials determine whether the inclusion of a moderate and a
high dose of the test-product in the diet affects feed intake, faeces consistency, as indicators of digestibility and general state of health. Weight gain is also measured as a first, very rough estimate of nutritional value (van Weerden, 1979).

The third step is the evaluation programme, determination of digestibility. This is to a great extent, a determination of the nutritional value of the product under test. After the results of the first three phases of the feed assay programme have become available, a reasonably reliable prediction of nutritional value can be given, provided the test product does not contain specific negative factors not discovered in the acceptability and digestibility trials (van Weerden, 1979).

The prediction is verified in the last, fourth step: the comparative feeding experiments. In these experiments, the test product replaces part or all of the usual high-protein components in the diet in order to find out how it affects weight gain, egg production, feed conversion efficiency, and product quality. Phase two (acceptability) and especially phase four (comparative feeding) allow inclusion of toxicological determinations, because the target animals are consuming moderate to high levels of the test product over a prolonged period (van Weerden, 1979). Due to the limited resources available, this study restricted the initial animal feeding trials to analysis, acceptability and palatability trials. In addition the study used broilers due to their short growth period, easily measurable growth parameters and low cost of production.
1.3 Problem statement and justification

Nutrient enrichment of agro-industrial waste through biological techniques is an exploitable avenue of improving livestock productivity by providing alternative feed supplemenation and/or lowering feed cost. Cirio Del Monte, a pineapple planting and processing company based in Thika, Kenya, produces 1500 tons of pineapple daily translating to approximately 750 tons of PW daily which is used mostly in the plantation fields as natural fertilizer (Cirio Del Monte, 2002). Much of this waste may be converted to more useful by-products if a better understanding of this biological nutrient enrichment technology is obtained.

This study investigated the potential use of fungi; *A. niger* and *T. viride* to enhance the nutrient content of PW by fermentation method and asses the potential use of such nutrient enhanced PW as feed supplement in poultry diet. It is hoped that the results shall provide more knowledge on the subject of fermentation of PW to enrich their nutrient content and the possibility of its use as feed in livestock.

The need for this study can be demonstrated since Kenya is an agricultural country with more than 80% of the population depending on agriculture and livestock production for subsistence, employment, income and other basic needs. Therefore, the necessity for exploring unconventional means of feed production cannot be over-looked as an additional strategy towards food security. However the application of this strategy has not been evaluated in Kenya and therefore a better understanding of this technology was required.
1.4 Research question
Can PW be nutritionally enhanced by fungal fermentation to provide an alternative feed source for poultry farmers to improve productivity?

1.5 Hypothesis
H₀: PW cannot be nutritionally enhanced by fungal fermentation to provide an alternative feed source for poultry farmers.

H₁: PW can be nutritionally enhanced by fungal fermentation to provide an alternative feed source for poultry farmers.

1.6 Objectives
1.6.1 General objective
The main objective of the study is to investigate whether fungi; *Aspergillus niger*, and *Trichoderma viride* can upgrade the nutritional value of PW for use as poultry feed.

1.6.2 Specific objectives
i) To evaluate the use of fungi; *Aspergillus niger* and *Trichoderma viride* for sustainable improvement in biomass content of PW through fermentation.

ii) To compare fermentation methods; SSF and LSF to determine suitability as appropriate methods for sustainable enhancement of nutrient content of PW.

iii) To investigate the conversion efficiency of such upgraded PW as feeds in poultry.
CHAPTER TWO
MATERIALS AND METHODS

2.1 Study site
The laboratory assays were conducted in the analytical chemistry research laboratories of the Department of Chemistry at Kenyatta University, Nairobi and the Microbiology Laboratory at Mt. Kenya University (MKU), Thika. The chicken feeding trials were conducted in a wooden chicken house located within the premises of MKU.

2.2 Fungal fermentation of pineapple waste

2.2.1 Pineapple waste
The pineapple (*Ananas comosus*) waste peelings of estimated equal freshness and peeled on the same day were sourced from pineapple vendors at Thika Municipal Market (Madaraka market) collected in polythene bags, transported and stored in a refrigerator set at 5°C at MKU. The pineapples originated from a pineapple growing and processing plant, Cirio Delmonte Kenya Limited Company, located within Thika Municipality.

2.2.2 Fungi
Isolated pure fungal strains of *A. niger* and *T. viride* grown on SARBORAUD agar medium were sourced from the Department of Veterinary Pathology, Parasitology and Microbiology, University of Nairobi. Slants of the microbes were transported in tightly sealed vial bottles carried in a cooler box filled with ice cubes to the Microbiology Laboratory at MKU.
2.2.3 Preparation of culture media

The fungal spores were prepared with CZAPEK dox broth media, a liquid microbial culturing media composed of 30 g/l Sucrose, 3 g/l Sodium nitrate, 1 g/l Dipotassium phosphate, 0.5 g/l Magnesium sulphate, 0.5 g/l Potassium chloride, 0.01 g/l Ferrous sulphate. pH 7.3 ± 0.2 at 25°C. The liquid media was prepared as outlined in the manufacturers’ guide whereby 35 g of the media powder was poured into 1 liter of distilled water and the mixture boiled to dissolve the medium completely. The preparation was then sterilized by autoclaving at 121°C for 15 minutes, poured into sterile vials and allowed to cool.

2.2.4 Fungal culturing

Fungal spores from the isolated pure strains of A. niger and T. viride were re-inoculated using sterilized wire loops into vial containing the prepared CZAPEK dox broth media. The vials were then sealed and placed in an oven set at 37°C to provide optimum growth conditions for the fungi. Culturing was done for four days. The grown fungal spores were then maintained by placing the preparations in a refrigerator set at 5°C.

2.2.5 Preparation of inoculums

The inoculums used were composed of 2 % spore suspension of either A. niger or T. viride. The 2 % spore suspension were prepared according to Guerra et al. (1986) by pouring 2 ml of the fungal spores grown in the CZAPEK Dox broth media into 98 ml of distilled water contained in a conical flask and shaking well to mix thoroughly. The suspensions were prepared just prior to PW inoculation.
2.2.6 Pineapple waste treatment

2.2.6.1 Unfermented sample

A sample of unfermented pineapple waste (UFPW) was prepared as outlined by Guerra et al. (1986) with the omission of the inoculation stage. The fresh PW were cut into approximately one inch square pieces and dried in an oven set at 60°C for 72 hours. The dried sample was then cooled, ground into granules using an electric grinder and stored in a tightly sealed plastic container. The sealed container was then labeled and placed in an airtight safety box awaiting nutrient analysis.

2.2.6.2 Liquid state fermentation samples

Liquid state fermentation of the PW was carried out according to the procedures outlined by Guerra et al. (1986). Six 500 ml beakers each containing 90 g of PW and 210 ml of distilled water were sterilized in an autoclave at 121°C for 15 minutes then cooled. Three beakers were then each inoculated with 15 ml of 2% fungal spore suspension prepared with *A. niger* and another three beakers inoculated with 15 ml of 2% fungal spore suspension prepared with *T. viride*. The preparations were then agitated in a shaker at 100 rpm and later incubated in an oven at 37°C. The first three beakers containing *A. niger* were allowed to ferment for 48, 72, and 96 hours, respectively. The same was repeated for the other set containing *T. viride*. Agitation was repeated after every 24 hours. After fermentation, the mycelia were filtered using a plastic sieve and placed on aluminum trays in an oven set at 60°C with air circulation for 72 hours. After drying, the preparations were allowed to cool in the oven and then ground into fine granules using an electrical grinder.
2.2.6.3 Solid state fermentation samples

Solid state fermentation of the PW was carried out according to the procedures outlined by Guerra et al. (1986). Fresh PW were cut into approximately one inch square pieces and placed into six 500 ml glass beakers in 90 g portions and then sterilized in an autoclave set at 121°C for 15 minutes. The PW were then spread on foil paper trays in uniform layers of 1 cm thick under a semi covered glass hood allowing sufficient aeration. Three preparations were each inoculated with 15 ml of 2 % fungal spore suspension prepared with A. niger and the other three inoculated using spore suspension prepared with T. viride. Fermentation was done at the prevailing room temperatures. The first three portions of PW inoculated with A. niger were allowed to ferment for 48, 72, and 96 hours, respectively. The same was repeated for the other set containing T. viride. After fermentation, the mycelia were dried at 60°C in an oven with air circulation for 72 hours. After drying the preparations were cooled and ground into fine granules using an electrical grinder.

2.2.7 Nutrient content analysis

The nutrient content of the treated PW were determined quantitatively by proximate analysis and specific mineral analysis. Analysis was done in triplicate for each sample. Proximate analysis was conducted using the methods of AOAC (1998). Proximate analysis involved the determination of crude protein, ash (mineral), crude fiber, dry matter, ether extract (lipid) and nitrogen free extract (NFE) which includes monosaccharides, oligosaccharides and starch generalized as carbohydrate content.
The specific minerals were determined according to the method described by Okalebo, Gathua and Woomer (1993). An Atomic Absorption Spectrophotometer (AAS) and direct air-acetylene flame were used in the procedure. In these AAS measurements, a hallow cathode lamp was used where each specific lamp was selected for each element being measured. The treated PW samples were digested on a block digester using concentrated HNO₃ for 30 minutes, filtered and various concentrations made. The standards were diluted from commercially acquired standards and aspirated into the flame. Calibration curves were then plotted and the treated PW samples aspirated from which various concentrations of the different metal ions were calculated. The measurements were carried out in triplicate and the standard deviation for the measurement was 0.002.

2.2.7.1 Crude protein analysis

Crude protein content was determined by Kjedahl method according to methods of AOAC (1998). The principle of the method is as follows; nitrogen (protein) and other organic compounds are transformed into sulphate by acid digestion with concentrated sulphuric acid (H₂SO₄) and a catalyst (selenium, Se). When digestion is completed the acid sample solution is cooled, diluted with water and made strongly basic with sodium hydroxide (NaOH). This process releases ammonia (NH₃) which is distilled into boric acid solution (H₃BO₃). The H₃BO₃ solution is then titrated with standardized hydrochloric acid (N/70 HCl). From this titration the amount of nitrogen (N) is determined. The N content is multiplied by 6.25 (conversion factor) to establish the protein content in the sample.
The procedure involved preparing a digestion mixture by mixing 0.42 g of Se powder, 14 g of Lithium sulphate, LiSO₄. H₂O added to 350 ml of 30% Hydrogen peroxide, H₂O₂, and mixed well. A 420 ml portion of concentrated H₂SO₄ was then slowly added to the mixture while cooling in an ice bath. Once prepared the solution was stored in a sealed glass flask in a refrigerator set at 2°C until used. The treated PW samples were placed in separate clean, labeled digestion tubes of a block digester apparatus in portions of 0.3 g and 4.4 ml of the digestion mixture added to each tube. A reagent blank was also prepared. The samples were then digested for 2 hours at 360°C and then allowed to cool slowly. Exactly 25 ml of distilled water was added to each sample, the contents mixed well until no more sediment dissolved and allowed to cool. The final volume was then made up to 50 ml with water and mixed well.

After digestion the samples were subjected to steam distillation. A steam distillation apparatus (Hoskyn nitrogen still) was set up and steam passed through the apparatus for 30 minutes. The steam blank was checked for NH₃ content by collecting 50 ml of the distillate and titrating with N/70 HCl. Aliquots of 5 ml of the digested sample solutions were transferred to the reaction chamber of the distillation still and 10 ml of 40% NaOH added. Steam distillation was then carried out immediately into 5 ml of 1% H₃BO₃ contained in a distillation flask added with 4 drops of a mixed indicator (bromocresol green, methyl red, thymol blue in 100 ml ethanol). Distillation was continued for 2 minutes from the time the indicator turned green. The distillate (NH₃) was then collected and titrated with N/70 HCl with the end point determined when the distillate turned from green through grey to a definite pink. The amount of N/70 HCl required was noted.
A blank determination was run by digesting the blank reagent sample, distilling and titrating as above. The amount of N/70 HCl required for the blank was then subtracted from the readings obtained for the other samples to give a corrected volume of N/70 HCl. The quantity of HCl required for titration was a measure of the NH$_3$ distilled into H$_3$BO$_3$ which in turn was a measure of the N in the sample. The amount of N in the sample was multiplied by a conversion factor of 6.25 to obtain the percentage protein in the sample as shown in the following equation.

\[
\text{% N in the sample} = \frac{\text{Corrected ml of N/70 HCl} \times 0.2}{\text{Weight of sample}}
\]

\[
\text{% Protein} = \text{% N in the sample} \times 6.25
\]

### 2.2.7.2 Ash (mineral) analysis

The ash content was determined according to methods of AOAC (1998). The principle of the method involved igniting the sample at 550°C to burn off all organic material. The inorganic material or ash which did not volatilize at the temperature is left and weighed.

The procedure involved using three clean individually marked porcelain crucibles. The crucibles were placed in an oven set at 105°C for an hour. They were then removed, cooled in a desiccator, quickly weighed and the weights recorded. The treated PW samples were weighed into 10 g portions and added into the porcelain crucibles. The crucibles with the samples were then placed in a muffle furnace with temperatures set at 550°C for 8 Hours. The sample containing crucibles were then cooled to about 100°C in the oven before transferring to a desiccator for cooling down to room temperature. The
porcelain crucibles were quickly weighed one by one from the desiccator. The total ash content was then calculated as a percentage of the initial weight of the sample as shown in the following equation.

\[
\text{Percentage ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100
\]

where,

- \( W_1 \) = Weight of empty dry crucible
- \( W_2 \) = Weight of dry crucible containing the sample before ignition
- \( W_3 \) = Weight of crucible containing the sample after ignition

### 2.2.7.3 Crude fiber analysis

The crude fiber content was determined according to methods of AOAC (1998). The principle of the method is as follows; Materials which are digestible with weak acid or weak base solution are removed leaving crude fiber and ash which are collected in a filter as residue. The crude fiber is burned off in a furnace and the amount is found by difference in weight.

The procedure involved weighing the treated PW samples into three portions of 2 g each and placing them into individually marked pre-weighed clean dry 600 ml beakers. The beakers were then added with 150 ml of 0.125 M \( \text{H}_2\text{SO}_4 \) and the mixtures boiled for 30 minutes on a hot plate with occasional stirring. The boiled sample acid solution was then filtered through a Buchner funnel. The residues were washed with cold deionized water until acid free and then carefully transferred clean labeled 400 ml beakers. A few drops of
octanol and 150 ml of 0.22 M Potassium hydroxide, KOH were added to the residues. The mixtures were then heated for 30 minutes with constant stirring on a magnetic stirrer.

The contents in the beakers were later cooled and then filtered through a Buchner funnel with a 15cm no. 4 whatman filter paper. The filtrate was then washed several times with hot water and once with methylated spirit until free from KOH. The residues were then transferred to porcelain crucibles and dried at 105°C for 2 hour before being cooled in a desiccator. The sample containing crucibles were then weighed and the weights recorded. The sample containing crucibles were then heated in a muffle furnace set at 550°C for 2 hours. The crucibles were then cooled in a desiccator and weighed. The crude fiber content was then determined by difference in weight and expressed as a percentage of the original sample weight. Calculation was as shown below.

\[
\text{Percentage crude fiber} = \frac{W_1 - W_2}{W_3} \times 100
\]

where

\[W_1 = \text{Weight of crucible + residue before ignition}\]
\[W_2 = \text{Weight of crucible + residue after ignition}\]
\[W_3 = \text{Weight of original sample}\]

2.2.7.4 Dry matter analysis

The dry matter content was determined according to methods of AOAC (1998). The principle of the method is as follows: Dry matter is the difference between the percentage moisture in a sample and 100. The moisture in a sample is lost by volatilization caused by
heat. The percent is calculated by difference in the weight of a sample before and after heat treatment.

The procedure involved using three clean individually marked porcelain crucibles, which were placed in an oven set at 105°C for one hour and then cooled to room temperature in a desiccator. After cooling the crucibles were accurately weighed and the weights recorded. The treated PW samples were weighed into 2 g portions and placed into individual crucibles and the crucibles placed in an oven set at 105°C for eight hours to dry. The dried samples in crucibles were then placed in a desiccator and cooled to room temperature. The crucibles with dried samples were then accurately weighed and the weight recorded. Difference in the initial and final weights of the samples were estimated as the moisture content calculated as shown below. Dry matter content was calculated as difference from the moisture content as shown below.

\[
\% \text{ Moisture} = \frac{W_1 - W_2}{W_3} \times 100
\]

\[
\% \text{ Dry matter} = 100 - \% \text{ moisture}
\]

where

\( W_1 = \) Weight of Sample + crucible weight before drying

\( W_2 = \) Weight of Sample + crucible weight after drying

\( W_3 = \) Weight of Sample before drying

2.2.7.5 Lipid (ether extract) analysis

The lipid content was determined by soxhlet extraction method according to methods of AOAC (1998). The principle of the method is as follows: Ether is heated and volatilized
then condensed and allowed to pass through a sample carrying ether soluble materials along. The process is repeated over and over until no more extractable material remains in the sample. The ether extract contains all the substances in the sample that are soluble in ether. These include wax, resins, chlorophyll, sterols, carotene, phospholipids fatsoluble vitamins in addition to pure fats generally referred to as lipids.

The procedure involved weighing the treated PW samples into three 3 g portions and placing them in clean unused individual soxhlet thimbles for ether extraction. The thimbles were plugged with absorbent cotton wool and placed in a soxhlet extractor apparatus. Exactly 210 ml of Diethyl ether solvent was then added into a round bottom flask which was then connected to the continuous system of the soxhlet extraction apparatus. Extraction was performed for eight hours by heating the round bottom flask with moderate heat using a hot plate. After extraction, the solvent collected in the round bottom flask was emptied into a clean pre-weighed beaker. The solvent (ether) was allowed to evaporate and the beaker dried by heating in an oven set at 105°C for about 30 minutes. The beaker with the extracted lipid sample was then cooled in a desiccator, weighed and the weight recorded. The percentage lipid extracted was then calculated versus the initial sample amount as shown in the following equation.

\[
% \text{Lipid} = \frac{W_1 - W_2}{W_3} \times 100
\]

where

\( W_1 = \) Weight of beaker + extracted lipids
\( W_2 = \) Weight of beaker
\( W_3 = \) Weight of original sample
2.2.7.6 Carbohydrate analysis

The carbohydrate content was determined according to methods of AOAC (1998). The principle of the method is as follows; the NFE portion in a sample includes monosaccharides, oligosaccharides and starch. It also contains some hemicellulose and lignin dissolved by the acid-base treatment in crude fiber determination. The sum total of crude fiber and NFE gives the carbohydrate content of a sample.

The procedure involved determining the NFE content of the PW by subtracting the percentage of moisture, crude protein, ash, lipid and crude fiber from 100 %. Carbohydrate content was then calculated as shown below.

\[
\% \text{ NFE} = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ ash} + \% \text{ lipid} + \% \text{ crude fiber})
\]

\[
\% \text{ Carbohydrate} = \% \text{ NFE} + \% \text{ crude fiber}
\]

2.2.7.7 Calcium analysis

The procedure involved preparation of a stock calcium solution of 1000 ppm Ca by dissolving 2.497 g dry (100°C, 2 hours) calcium carbonate (CaCO₃) in minimum quantity dilute 1N HCl and made to 1 liter with distilled water. A working Ca solution of 500 ppm was made by diluting the Ca stock solution to 100 ml with distilled water. A stock potassium solution of 1000 ppm K was prepared by dissolving 1.907 g potassium chloride (KCl) in 1 liter of distilled water. A working K solution of 1000 ppm was then prepared by diluting 20 ml of the stock K solution into 200 ml with distilled water. A stock sodium solution, 1000 ppm Na was prepared by dissolving 2.541 g of dry sodium chloride (NaCl) in distilled water and making the final volume to 1 liter. A working Na
solution was prepared by diluting 20 ml of the stock Na solution to 200 ml with distilled water. Lanthanum chloride 0.15 %, LaCl₃.7H₂O, was prepared by dissolving 1.5 g LaCl₃.7H₂O in 1 liter of distilled water. 1N H₂SO₄ was prepared by dissolving 25 ml of concentrated H₂SO₄ to 1 liter with distilled water.

The working Ca solution of 500 ppm Ca was pipetted into four different 500 ml volumetric flasks in portions of 0.5, 10, 15, 20 and 30 ml to give standard solutions containing 0, 5, 10, 15, 20 and 30 ppm Ca, respectively. Each flask was then added with 25 ml of the 100 ppm K solution, 25 ml of the 50 ppm Na solution, 200 ml of the 0.15 % LaCl₃.7H₂O solution, 70 ml of 1 N H₂SO₄ and the final volume made to one liter with distilled water. The standard Ca solutions were then aspirated into the AAS and the results obtained used to plot a calibration curve of absorbance against standard concentration. Ca determination was done at a wavelength of 4227 Å and slit width of 0.07 nm using an air-acetylene flame. Exactly 10 ml of the treated PW digested as outlined in the digestion procedure 2.3.5.1 were poured into 50 ml volumetric flasks and 10 ml of 0.15 % LaCl₃.7H₂O solution added. The solution was made up to 50 ml with distilled water, shaken to mix and then aspirated on the same AAS as the standard Ca solutions. The solution concentrations for each treated PW and 2 blanks were determined from the graph of absorbance against standard concentration. The mean blank value was subtracted from the unknowns to get the corrected concentrations (c).

\[
\% \text{ Ca in sample} = \frac{c \times 0.025}{W}
\]

where \( c \) = corrected concentration for sample solution (in ppm Ca)

\( W \) = weight of sample taken (0.3 g)
2.2.7.8 Phosphorous analysis

Reagents for phosphorous determination were prepared as follows: A standard phosphorous stock solution 1000 ppm P was prepared by weighing 1.0967 g of oven dry potassium phosphate (KH$_2$PO$_4$) and dissolve with distilled water. The solution was then made up to 250 ml with distilled water (1 ml = 1 mg P). A working P solution 10 ppm P was prepared by diluting 10 ml of the above stock P solution (1000 ppm P) solution to 1 liter with distilled water.

One liter of ammonium molybdate / ammonium vanadate mixed reagent was prepared using 140 ml concentrated nitric acid (HNO$_3$), 1g ammonium vanadate and 20 g ammonium molybdate. The mixed reagent was prepared as follows; Ammonium molybdate was dissolved in 400 ml of distilled water and warmed to about 50 °C. The ammonium vanadate was then dissolved in about 300 ml of boiling distilled water, the solution cooled and the HNO$_3$ added slowly. The ammonium molybdate solution was then added gradually into the ammonium vanadate / HNO$_3$ mixture while stirring and the mixture diluted to 1 liter with distilled water. Paranitrophenol 0.5 % w/v was prepared by weighing 0.5 g p-nitrophenol and dissolving in distilled water then making the solution up to 100 ml with water. 1 N HNO$_3$ was prepared by diluting 63 ml conc. HNO$_3$ to 1 liter with distilled water. Aqueous ammonia, 6 N NH$_3$ was prepared by diluting 420 ml of concentrated NH$_3$ solution to 1 liter with distilled water.

Exactly 10 ml of the digested PW sample solutions (digestion procedure 2.5.1.2) were pipetted into 50 ml volumetric flask. A portion of 0.2 ml of 0.5 p-nitrophenol indicator
solution was then added to each flask. To make the solution alkaline (yellow colour), 6 N NH₃ solution was added by drop wise addition with gentle shaking. In each flask, 5 ml of the ammonium molybdate / ammonium vanadate mixed reagent was then added. The mixtures were made up to 50 ml with distilled water and mixed well. The flasks were kept for 30 minutes and the absorption of the solution measured using an AAS at 400 nm wavelength setting.

A calibration curve of absorbance against standard concentration of P content was prepared by pipetting 0, 5, 10, 15, 20 and 25 ml of the standard 10 ppm P solution into 50 ml volumetric flask representing 0, 1, 2, 3, 4 and 5 ppm P respectively. The vanadomolybdate yellow colour was developed in the standard P solutions by the addition of p-nitrophenol indicator, NH₃ solution and HNO₃ as above. The solution concentrations for each treated PW and 2 blanks were determined from the graph of absorbance against standard concentration. The mean blank value was subtracted from the unknowns to get the corrected concentrations (c). Percentage phosphorous content in the treated PW were calculated as shown below.

\[
\% \text{ P in sample} = \frac{c \times 0.025}{W}
\]

where

\( c = \) the corrected concentration for sample solution (in ppm P)

\( W = \) the weight of sample taken (0.3 g)
2.2.7.9 Magnesium analysis

A stock Magnesium solution of 1000 ppm Mg was prepared by weighing accurately 1.000 g of specpure Mg rod and dissolving into 30 ml of 1:1 HNO₃. The final volume was then made up to one liter with distilled water. A working magnesium solution of 50 ppm Mg was prepared by diluting 10 ml of the above stock Mg solution to 200 ml with distilled water. 1N H₂SO₄ was prepared by dissolving 25 ml of concentrated H₂SO₄ to 1 liter with distilled water. Portions of 50, 40, 30, 20, 10, 5 and 0 ml of 50 ppm Mg solution were separately diluted to 1 liter by adding 140 ml of 1N H₂SO₄ to each flask before making up to 1 liter with distilled water. This gave standard solutions containing 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 and 0 ppm Mg, respectively.

Exactly 5 ml of the treated PW digested as outlined in the digestion procedure 2.5.1.1 were poured into 50 ml volumetric flasks and the solution was made up to 50 ml with distilled water and then content shaken to mix. The sample solutions and the standard Mg solutions were then aspirated on the same AAS. Mg content was determined at a wavelength of 2852 Å nm, slit width of 0.1 nm and an air-acetylene flame. A calibration graph of absorbance against standard concentration of the Mg content in the standard solutions was plotted. The solution concentrations for each treated PW and 2 blanks were determined from the graph of absorbance against standard concentration. The mean blank value was subtracted from the unknowns to get the corrected concentrations (c). The percentage Mg content in the sample was calculated as below.

\[
\% \text{ Mg in sample} = \frac{c \times 0.025}{W}
\]

where
The study used 50 unsexed broilers chicks grown over a 42 days experimental period. The broilers were purchased from a commercial hatchery, randomly selected and randomly divided into five uniform live weight groups of ten broilers per group. Each group was placed in a specific cage and fed on a specific diet. All the broilers weighed an average of 35 g when the experiment started.

The broilers were housed in a wooden chicken house measuring 2 m x 4 m x 1.2 m and divided into eight 1 m x 1 m cages using wire mesh. The house was rectangular in shape; open sided to allow natural ventilation and having an east-west orientation to minimize the amount of sunlight entering the house directly. The longer walls were made of wood on the lower (0.7 m) half and wire mesh on the upper (0.5 m) half while the sides were made with wood. The meshed walls were covered with old sack cloths to act as curtains against extreme sunlight and cold. The roof was made of iron sheets and the floor wooden. The floor was covered with wood shavings and old sack cloths. The house was raised about 1 meter above the ground on wooden stilts and the vegetation around the house cleared to guard against rodents and other vectors of poultry diseases. Prior to introducing the chicken the house, equipments and the area around the house was disinfected. This was repeated weekly.
2.3.2 Diets

2.3.2.1 Diet formulation

One reference and four test diets were prepared for the study. The diets were formulated from standard commercial broiler feed (SCBF) and fermented PW (FPW). PW fermented with *A. niger* and *T. viride* for 72 hours and 96 hours were chosen to formulate the test diets A, B, D and E based on their superior proximate composition (specifically protein content) as reported on Table 2. The FPW were prepared by SSF as outlined in section 2.2.6.3 and dried in a roofed wooden shade with open sides to allow air flow. The wastes were later crushed using a grinder and sieved with a 12 mm sieve ready for diet formulation.

Diet A was formulated using PW fermented with *A. niger* for 72 hours mixed in the ratio of 1:1 with SCBF. Diet B was formulated using PW fermented with *T. viride* for 96 hours mixed in the ratio of 1:1 with SCBF. The diets were formulated for the first phase of the feeding trials with SCBF comprising of starter broiler chicken feed and the second phase of the feeding trials with finisher broiler chick feed. Diet C comprised of 100 % SCBF also divided into starter broiler chick feed for phase one of the feeding trials and finisher broiler chick feed for phase two of the feeding trials. Diet C represented the reference diet. Diets D and E were prepared from 100 % PW fermented with *A. niger* for 72 hours and *T. viride* for 96 hours, respectively.
Table 1: Formulation of reference and test diets

<table>
<thead>
<tr>
<th>Content</th>
<th>Diet composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Standard commercial broiler feed</td>
<td>50</td>
</tr>
<tr>
<td>PW fermented with <em>A. niger</em> for 72 hrs</td>
<td>50</td>
</tr>
<tr>
<td>PW fermented with <em>T. viride</em> for 96 hrs</td>
<td>-</td>
</tr>
</tbody>
</table>

The nutrient content values of the reference and test diets were determined by proximate and specific mineral analysis procedures outlined in section 2.2.7.

2.3.3 Experimental design

2.3.3.1 Allocation of diets to chickens
The feeding trials were conducted over a 42 day period divided into two phases of starter phase (day 1 - 28) and finisher phase (day 29 - 42). The two phases were meant to accommodate the broiler feeding regimen which comprises a starter and finisher marsh. The chickens were fed on one specific diet throughout the feeding trials. Four test diets and the reference diet were allocated randomly to five cages of ten chickens per cage.

2.3.3.2 Parameters considered
The feeding trials were designed to monitor efficacy of formulated test diets as animal feed supplement. The parameters observed on the animals included feed acceptability and palatability, feed intake, live body weight gain, general state of health and feed conversion efficiency. Post-mortem studies were done on randomly selected animals per test diet to assess for feed related pathology and cause of death for animals that died during the trials.
2.3.3.3 Management

The animals were fed *ad libitum* and clean drinking water made available at all times. Special infra red lighting was provided for lighting and to keep the house temperatures within the appropriate levels. The house temperature was constantly monitored and maintained between 30 - 35°C during the first 3 weeks and 25 – 30°C during the last three weeks. The birds were vaccinated against infectious bronchitis (IB), Newcastle disease and infectious bursal disease (gumboro disease) as prescribed in the kenchic broiler management manual. A vitamin, mineral and antibiotic premix (Pfizer – 1000) was included in the daily rations for all the birds.

The cages were cleaned daily and feeding done twice a day at 09.00 am and 18.00 pm. The animals were weighed at the commencement of the feeding trials and then once every week for six weeks. The feeds under study were always weighed before being served to the birds and unconsumed feeds collected from each cage and weighed before fresh feed was served to determine the amount of feed consumed by the animals per day.

2.4 Statistical analysis

Data from nutrient analysis and feeding trials were subjected to analysis for significant differences by the analysis of variance procedure. A one way analysis of variance (ANOVA) and post ANOVA was used. Significance was accepted at *p* < 0.05. SPSS version II statistical computer package was used to analyze data from the experiments.
CHAPTER THREE
RESULTS

3.1 Nutrient composition of treated pineapple waste

Table 2 shows the proximate composition of the treated PW samples.

3.1.1 Crude protein content

Results for LSF using fungi *A. niger* and *T. viride* indicate that: the FPW had more crude protein than the UFPW for the 48, 72 and 96 hours fermentation periods; the FPW did not change from 48 to 96 hours.

Results for SSF method using fungi *A. niger* and *T. viride* indicate that: the FPW had more crude protein than the UFPW for the three fermentation periods; the FPW had more crude protein at 72 and 96 hours than at 48 hours fermentation period; the FPW had more crude protein at 72 hours than at 96 hours fermentation period using *A. niger*; the FPW had more crude protein at 96 hours than at 72 hours fermentation period using *T. viride*.

Results for SSF and LSF methods using fungi *A. niger* and *T. viride* indicate that: the FPW had more crude protein in the SSF method than the LSF method for the 72 and 96 hours fermentation periods; the results did not differ for the 48 hours fermentation period.

3.1.2 Ash content

Results for LSF method using fungi *A. niger* and *T. viride* indicate that: the FPW did not change when compared to the UFPW for the 48 and 72 hours fermentation periods using
A. niger; the FPW had more ash than the UFPW at 96 hours fermentation period using A. niger; the FPW did not change when compared to the UFPW for the three fermentation periods using T. viride; the FPW did not change at 72 hours when compared with the results at 48 hours fermentation period; the FPW had more ash at 96 hours than at 48 and 72 hours fermentation periods using A. niger; the FPW did not change at 96 hours when compared with the results at 48 and 72 hours fermentation periods using T. viride;

Results for SSF method using fungi A. niger and T. viride indicate that: the FPW had more ash than the UFPW for the three fermentation periods; the FPW did not change at 72 and 96 hours when compared with the results at 48 hours fermentation period; the FPW did not change at 96 hours when compared with the results at 72 hours fermentation period.

Results for SSF and LSF methods using fungi A. niger and T. viride indicate that: the FPW had more ash in the SSF method than the LSF method for the three fermentation periods using T. viride; the FPW had more ash in the SSF method than the LSF method for the 48 hours fermentation period using A. niger; the results did not differ for the 72 and 96 hours fermentation periods using A. niger.

3.1.3 Crude fiber content

Results from LSF method using fungi A. niger and T. viride indicate that: the FPW had more crude fiber than the UFPW for the 48, 72 and 96 hours fermentation periods; the FPW crude fiber decreases from 48 to 96 hours.
Results for SSF method using the fungi *A. niger* and *T. viride* indicate that: the FPW had more crude fiber than the UFPW for the 48 hours fermentation period using *A. niger*; the FPW had less crude fiber than the UFPW at 72 and 96 hours fermentation periods using *A. niger*; the FPW had less crude fiber than the UFPW at the three fermentation periods using *T. viride*; the FPW crude fiber decreased from 48 to 96 hours.

Results for SSF and LSF methods using the fungi *A. niger* and *T. viride* indicate that: the FPW had less crude fiber in the SSF method than the LSF method for the three fermentation periods.

3.1.4 Dry matter content

Results for LSF method using fungi *A. niger* and *T. viride* indicate that: the FPW had more dry matter than the UFPW for the 48, 72 and 96 hours fermentation periods; the FPW did not change at 72 hours when compared with the results at 48 hours fermentation period; the FPW had more dry matter at 96 hours than at 48 and 72 hours fermentation periods using *A. niger*; the FPW did not change at 96 hours when compared with the results at 48 and 72 hours fermentation period using *T. viride*.

Results for SSF method using fungi *A. niger* and *T. viride* indicate that: the FPW had more dry matter than the UFPW for the three fermentation periods; the FPW did not change at 72 hours when compared with the results at 48 hours fermentation period; the FPW did not change at 96 hours when compared with the results at 48 and 72 hours
fermentation period using *A. niger*; the FPW had more dry matter at 96 hours than at 48 and 72 hours fermentation periods using *T. viride*.

Results for SSF and LSF methods using fungi *A. niger* and *T. viride* indicate that: the FPW had more dry matter in the SSF method than the LSF method for the 48 hours fermentation period using *A. niger*; the FPW had more dry matter in the SSF method than the LSF method for the 72 and 96 hours fermentation periods using *T. viride*; the results did not differ for the 48 hours fermentation period using *T. viride* and for the 72 and 96 hours fermentation periods using *A. niger*.

### 3.1.5 Carbohydrate content

Results for LSF method using fungi *A. niger* and *T. viride* indicate that: the FPW had more carbohydrate than the UFPW for the 48, 72 and 96 hours fermentation periods; the FPW did not change at 72 and 96 hours when compared with the results at 48 hours fermentation period; the FPW did not change at 96 hours when compared with the results at 72 hours fermentation period.

Results for SSF method using fungi *A. niger* and *T. viride* indicate that: the FPW had more carbohydrate than the UFPW for the 48 hours fermentation period using *A. niger*; the FPW did not change after 72 and 96 hours fermentation periods when compared to the UFPW using *A. niger*; the FPW did not change after 48, 72 and 96 hours fermentation periods when compared to the UFPW using *T. viride*; the FPW had less carbohydrate at 72 and 96 hours when compared with the results at 48 hours fermentation period using *A. niger*.
*niger*; the FPW did not change at 48, 72 and 96 hours fermentation periods using *T. viride*; the FPW did not change at 96 hours when compared with the results at 72 hours fermentation period using *A. niger*.

Results for SSF and LSF methods using fungi *A. niger* and *T. viride* indicate that: the FPW had less carbohydrate in the SSF method than the LSF method for the three fermentation periods using *T. viride*; the FPW had less carbohydrate in the SSF method than the LSF method for 72 and 96 hours fermentation periods using *A. niger*; the results did not differ at 48 hours fermentation period using *A. niger*. 
Table 2: Proximate composition of treated PW samples (g / 100 g)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Fermentation Method</th>
<th>Fungi</th>
<th>Unfermented 0 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>LSF</td>
<td>A. niger</td>
<td>3.69 ± 0.05</td>
<td>5.04 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.37 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.00 ± 0.42&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td>A. niger</td>
<td></td>
<td>4.84 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.56 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.58 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. viride</td>
<td></td>
<td>4.53 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.28 ± 0.14&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>8.89 ± 0.28&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. niger</td>
<td></td>
<td>4.44 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.32 ± 0.01&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>9.04 ± 0.16&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>LSF</td>
<td>A. niger</td>
<td>2.61 ± 0.23</td>
<td>3.04 ± 0.25&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.42 ± 0.02</td>
<td>5.06 ± 0.03&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td>A. niger</td>
<td></td>
<td>2.51 ± 0.38&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.03 ± 0.00&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.79 ± 0.84&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. viride</td>
<td></td>
<td>5.14 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.18 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.79 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. niger</td>
<td></td>
<td>4.95 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.64 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.48 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td>T. viride</td>
<td></td>
<td>18.01 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.96 ± 0.01&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>13.26 ± 0.01&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. niger</td>
<td></td>
<td>18.78 ± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.37 ± 0.02&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>17.93 ± 0.01&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td>T. viride</td>
<td></td>
<td>14.09 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.78 ± 0.01&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>2.49 ± 0.01&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.80 ± 0.10</td>
<td></td>
<td>9.09 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.04 ± 0.04&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>7.59 ± 0.08&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>LSF</td>
<td>A. niger</td>
<td>82.13 ± 0.46</td>
<td>88.12 ± 0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>88.46 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.15 ± 0.20&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td>A. niger</td>
<td></td>
<td>89.36 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.88 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>90.5 ± 0.58&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. viride</td>
<td></td>
<td>89.74 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.81 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.39 ± 0.27&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.83 ± 1.12</td>
<td></td>
<td>87.24 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.16 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.84 ± 0.41&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry matter</td>
<td>LSF</td>
<td>A. niger</td>
<td></td>
<td>80.04 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.67 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.09 ± 0.30&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td>A. niger</td>
<td></td>
<td>82.01 ± 0.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>80.29 ± 0.47&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>82.15 ± 0.79&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. viride</td>
<td></td>
<td>80.07 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.36 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>76.71 ± 0.05&lt;sup&gt;cdef&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>77.85 ± 0.08</td>
<td></td>
<td>74.20 ± 0.85</td>
<td>74.20 ± 0.85</td>
<td>75.32 ± 0.33</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 3) for different determinations. <sup>a</sup> indicates significant difference of values between 0 and 48 hours; <sup>b</sup> indicates significant difference of values between 0 and 72 hours; <sup>c</sup> indicates significant difference of values between 0 and 96 hours; <sup>d</sup> indicates significant difference of values between 48 and 72 hours; <sup>e</sup> indicates significant difference of values between 48 and 96 hours; <sup>f</sup> indicates significant difference of values between 72 and 96 hours. <sup>*</sup> indicates significant difference of values obtained by the same fungi under different fermentation methods at the same fermentation period. Significant difference determined at P < 0.05.
Table 3 shows the mineral composition (mg/g) of treated PW samples.

3.1.6 Calcium content

Results for LSF using fungi *A. niger* and *T. viride* indicate that: the FPW had less calcium than the UFPW for the 48, 72 and 96 hours fermentation periods; the FPW had more calcium at 72 and 96 hours than at 48 hours fermentation period using *A. niger*; the FPW had more calcium at 72 hours than at 96 hours fermentation period using *A. niger*; the FPW did not change at 72 hours when compared with the results at 48 hours fermentation period using *T. viride*; the FPW had less calcium at 96 hours than at 48 hours fermentation period using *T. viride*; the FPW did not change at 96 hours when compared with the results at 72 hours fermentation period using *T. viride*.

Results for SSF method using fungi *A. niger* and *T. viride* indicate that: the FPW had more calcium than the UFPW for the 48 hours fermentation period using *A. niger*; the FPW did not change when compared to the UFPW for the 72 and 96 hours fermentation periods using *A. niger*; the FPW did not change when compared to the UFPW for the 48 and 72 hours fermentation periods using *T. viride*; the FPW had less calcium than the UFPW for the 96 hours fermentation period using *A. niger*; the FPW had less calcium for the 72 and 96 hours than for the 48 hours fermentation period using *A. niger*; the FPW had more calcium for 96 hours than for the 72 hours fermentation period using *A. niger*; the FPW did not change at 72 hours when compared with the results at 48 hours fermentation period using *T. viride*; the FPW had less calcium for the 96 hours than for the 48 and 72 hours fermentation periods using *T. viride*.
Results for SSF and LSF methods using fungi *A. niger* and *T. viride* indicate that: the FPW had more calcium in the SSF method than the LSF method for the 48 hours fermentation period using *A. niger*; the FPW had more calcium in the SSF method than the LSF method for the 72 hours fermentation period using *T. viride*; the results did not differ for the 72 and 96 hours fermentation periods using *A. niger* and for the 48 and 96 hours fermentation periods using *T. viride*.

### 3.1.7 Phosphorous content

Results for LSF method using fungi *A. niger* and *T. viride* indicate that: the FPW phosphorous content did not change when compared to the UFPW for the three fermentation periods; the FPW did not change at 72 and 96 hours when compared with the results at 48 hours fermentation period; the FPW did not change at 96 hours when compared with the results at 72 hours fermentation period.

Results for SSF method using fungi *A. niger* and *T. viride* indicate that: the FPW phosphorous content did not change when compared to the UFPW for the three fermentation periods; the FPW did not change at 72 and 96 hours when compared with the results at 48 hours fermentation period; the FPW did not change at 96 hours when compared with the results at 72 hours fermentation period.

Results for SSF and LSF methods using fungi *A. niger* and *T. viride* indicate that: the FPW had more phosphorous in the SSF method than the LSF method for the 72 and 96 hours fermentation periods using *A. niger*; the FPW had more phosphorous in the SSF
method than the LSF method for the 72 hours fermentation period using *T. viride*; the results did not differ for the 48 and 96 hours fermentation periods using *T. viride* and for the 48 hours fermentation period using *A. niger*.

### 3.1.8 Magnesium content

Results for LSF using fungi *A. niger* and *T. viride* indicate that; the FPW had less magnesium than the UFPW for the three fermentation periods; the FPW did not change at 72 hours when compared with the results at 48 hours fermentation period using *A. niger*; the FPW had more magnesium at 96 hours than at 48 and 72 hours fermentation periods using *A. niger*; the FPW had less magnesium at 72 hours than at 48 hours fermentation period using *T. viride*; the FPW had more magnesium at 96 hours than at 48 and 72 hours fermentation periods using *T. viride*.

Results for SSF using fungi *A. niger* and *T. viride* indicate that; the FPW had more magnesium than the UFPW for the 48 and 96 hours fermentation periods; the FPW had less magnesium than the UFPW for 72 hours fermentation period; the FPW had less magnesium at 72 hours than at 48 hours fermentation period; the FPW had more magnesium at 96 hours than at 48 and 72 hours fermentation periods.

Results for SSF and LSF methods using fungi *A. niger* and *T. viride* indicate that; the FPW had more magnesium in the SSF method than the LSF method for the three fermentation periods.
### Table 3: Minerals composition of treated PW (mg/g)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Fermentation Method</th>
<th>Fungi</th>
<th>Unfermented 0 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>LSF</td>
<td><em>A. niger</em></td>
<td>12.83 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. viride</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td><em>A. niger</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. viride</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.54 ± 0.01</td>
<td>10.32 ± 0.04</td>
<td>11.99 ± 0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.50 ± 0.14</td>
<td>9.82 ± 0.03</td>
<td>2.63 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorous</td>
<td>LSF</td>
<td><em>A. niger</em></td>
<td>1.22 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. viride</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td><em>A. niger</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. viride</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.70 ± 0.02</td>
<td>1.92 ± 0.12</td>
<td>2.55 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.69 ± 0.03</td>
<td>1.99 ± 0.05</td>
<td>1.87 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>LSF</td>
<td><em>A. niger</em></td>
<td>1.15 ± 0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. viride</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSF</td>
<td><em>A. niger</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. viride</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20 ± 0.00</td>
<td>1.05 ± 0.00</td>
<td>1.47 ± 0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 3) for different determinations. *a* indicates significant difference of values between 0 and 48 hours; *b* indicates significant difference of values between 0 and 72 hours; *c* indicates significant difference of values between 0 and 96 hours; *d* indicates significant difference of values between 48 and 72 hours; *e* indicates significant difference of values between 48 and 96 hours; *f* indicates significant difference of values between 72 and 96 hours. *g* indicates significant difference of values obtained by the same fungi under different fermentation methods at the same fermentation period. Significant difference determined at P < 0.05.
3.2 Results of feeding trials with chicken (broilers)

3.2.1 Nutrient composition of reference and test diets

Table 4 shows the nutrient composition of the reference and test diets.

Results show that: diets A, B, D and E have lower protein, ash, crude fiber, lipid, and higher carbohydrate content than the reference diet (Diet C). Diet A, B, and C had similar levels of calcium, and magnesium which were lower than those of diets D and E. Diets A, B, C, D, and E had similar levels of phosphorus and dry matter.

Table 4: Nutrient composition of reference and test diets (g/100g)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
<th>Diet D</th>
<th>Diet E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starter</td>
<td>Finisher</td>
<td>Starter</td>
<td>Finisher</td>
<td>Starter</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>5.53</td>
<td>5.36</td>
<td>5.61</td>
<td>6.68</td>
<td>7.79</td>
</tr>
<tr>
<td>Dry matter</td>
<td>88.97</td>
<td>89.58</td>
<td>88.86</td>
<td>88.90</td>
<td>89.45</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>60.98</td>
<td>61.83</td>
<td>59.25</td>
<td>59.49</td>
<td>47.89</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1.33</td>
<td>1.50</td>
<td>1.45</td>
<td>1.42</td>
<td>2.86</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.38</td>
<td>0.46</td>
<td>0.27</td>
<td>0.46</td>
<td>0.38</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.0042</td>
<td>0.0012</td>
<td>0.0024</td>
<td>0.0020</td>
<td>0.0028</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.12</td>
<td>1.11</td>
<td>0.97</td>
<td>1.06</td>
<td>0.88</td>
</tr>
</tbody>
</table>
3.2.2 Mortality

All the broilers fed on diets D and E died within the first week of the feeding trials and are thus no feeding trial results are represented in Tables 4 and 5. The deaths were found to be as a result of difficulty in digesting the feed causing poor motility and blockage of the gut. No mortalities were recorded with diets A, B and C.

3.2.3 Feed intake and feed conversion

Table 5 shows the feed intake and feed conversion ratio (FCR) of broilers fed on test diets A, B and C. Results of the feeding trials on broilers indicate that; feed intake of broilers fed on diet C was significantly (P < 0.05) better than the feed intake of diets A and B for weeks 2, 3, 5 and 6; the feed intake of broilers fed on diet A was significantly (P < 0.05) better than that of diet B for weeks 5 and 6; the feed intake of the broilers progressively increased each week with all the diets; the feed conversion ratio (FCR) of broilers fed on diets C was significantly (P < 0.05) better than FCR of broilers fed on diets A and B in the whole study period; the FCR of broilers fed on diets A and B were not significantly (P > 0.05) different for weeks 2, 3, 5 and 6.
Table 5: Feed intake and feed conversion ratio of broilers fed on test diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Average daily feed intake per bird per week (g)</th>
<th>Feed conversion ratio (Feed intake / weight gained per bird per week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.86 ± 1.42&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>30.12 ± 4.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>8.06 ± 1.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.05 ± 3.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>9.59 ± 1.33</td>
<td>40.03 ± 5.29</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 3) for different determinations in the daily feed intake. <sup>a</sup> indicates significant difference of values between diet A and B; <sup>b</sup> indicates significant difference of values between diet A and C; <sup>c</sup> indicates significant difference of values between diet B and C; <sup>*</sup> indicates no significant difference of values obtained with same test diet at different feeding stages. Significant difference determined at P < 0.05.

3.2.4 Live body weight

Table 6 shows the body weight and weight gain of broilers fed on test diets A, B and C. Results of the feeding trials on broilers indicate that; the body weight of broilers fed on diet C was significantly (P < 0.05) better than the body weight of diets A and B for weeks 2, 3, 5 and 6; the body weight of broilers fed on diets A and B were not significantly (P > 0.05) different for weeks 2, 3, 5 and 6; the body weight of the broilers progressively increased each week with all the diets.
Table 6: Cumulative body weight and weight gain of broilers fed on test diets

Cumulative body weight per bird per week (g)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>58.99 ± 0.84</td>
<td>105.95 ± 2.60</td>
<td>200.60 ± 5.06</td>
<td>315.90 ± 9.19</td>
<td>605.70 ± 18.33</td>
<td>732.40 ± 12.58</td>
</tr>
<tr>
<td>B</td>
<td>58.96 ± 1.91</td>
<td>114.06 ± 4.12</td>
<td>199.90 ± 7.82</td>
<td>342.80 ± 10.82</td>
<td>624.30 ± 14.07</td>
<td>743.60 ± 13.57</td>
</tr>
<tr>
<td>C</td>
<td>103.40 ± 3.80</td>
<td>267.40 ± 11.12</td>
<td>481.90 ± 13.03</td>
<td>890.00 ± 37.86</td>
<td>1267.00 ± 133.92</td>
<td>1700.00 ± 44.72</td>
</tr>
</tbody>
</table>

Body weight gain per bird per week (g)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23.99</td>
<td>46.96</td>
<td>94.65</td>
<td>115.30</td>
<td>289.80</td>
<td>126.70</td>
</tr>
<tr>
<td>B</td>
<td>23.96</td>
<td>55.10</td>
<td>85.84</td>
<td>142.90</td>
<td>281.50</td>
<td>119.30</td>
</tr>
<tr>
<td>C</td>
<td>68.40</td>
<td>164.00</td>
<td>214.50</td>
<td>408.10</td>
<td>377.00</td>
<td>433.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 3) for different determinations in the cumulative body weight. \( a \) Indicates significant difference of values between diet A and B; \( b \) Indicates significant difference of values between diet A and C; \( c \) Indicates significant difference of values between diet B and C; * Indicates no significant difference of values obtained with same test diet at different feeding stages. Significant difference determined at \( P < 0.05 \)
CHAPTER FOUR
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

Nutrient enrichment of PW through fungal fermentation was achieved in this study. The study targeted the increase of crude protein and decrease of crude fiber as a means of improving the nutritive value of PW for use as animal feed supplement. Most agro-industrial wastes, such as PW are low in protein content and high in fiber content specifically cellulose, hemicellulose and simple sugars (Chaiprasert, Bhumiratana and Tanticharoen, 2001). For such industrial solid waste material to be useful in livestock feeding it should undergo a nutrient enriching process especially protein enrichment (Chaiprasert, Bhumiratana and Tanticharoen, 2001). Microbial fermentation has been proposed as an efficient method of nutrient improvement of agro-industrial waste (Duru and Uma, 2003).

Results for LSF and SSF reported significantly (P < 0.05) higher crude protein content in PW fermented using the fungi A. niger and T. viride than in the UFPW for the 48, 72 and 96 hours fermentation periods. The post fermentation increase in crude protein content could be attributed to the possible secretion of some extra cellular enzymes (protein) such as amylases, xylanases and cellulases into the PW mash by the fermenting fungi in an attempt to make use of the carbohydrates in the mash as a carbon and energy source (Raimbault, 1998). A. niger has been reported to have high specific activity for cellulasces and hemicellulases (Howard et al., 2003). Additionally, T. viride and A. niger have found
use in the production of extra cellular enzymes including cellulase, amylase and xylanase (Nair, Sindhu and Shashidhar, 2008).

Fungi colonize substrates for utilization of available nutrients. They synthesize and excrete high quantities of hydrolytic extra cellular enzymes, which catalyze the breakdown of nutrients to products that enter the fungal mycelia across cell membrane to promote biosynthesis and fungal metabolic activities leading to growth (Raimbault, 1998). Additionally, increase in the growth and proliferation of fungal biomass in the form of SCP or microbial protein accounts for part of the increase in the protein content of the FPW.

These results are in line with earlier findings of Guerra et al. (1986), who reported significant (P < 0.05) increase in crude protein content in PW fermented for 72 and 96 hours by LSF using fungi, A. niger, T. viride and M. verrucaria. Similar findings have been reported with the same methods and fungi using substrates such as coffee pulp (Penaloza, 1985), cashew waste (Stamford et al., 1987), cassava waste (Pothiraj, Belaji and Eyini, 2006), wheat offal (Iyayi, 2004), maize offal (Iyayi and Aderolu, 2004; Iyayi, 2004), brewers dried grain (Iyayi and Aderolu, 2004; Iyayi, 2004), palm kernel meal (Iyayi and Aderolu, 2004) and rice bran (Iyayi and Aderolu, 2004; Oshoma and Ikenebomeh, 2005).

Fermentation of PW using fungi A. niger and T. viride recorded significantly (P < 0.05) more crude protein in the SSF method than the LSF method for the 72 and 96 hours.
fermentation periods. The difference was not significant at 48 hours fermentation period, though higher figures were recorded in SSF than LSF. PW is high in cellulose and hemicellulose which are known inducers of xylanase and cellulase enzyme production (Nair, Sindhu and Shashidhar, 2008). Strains of *A. niger* and *T. viride* have been reported to produce substantial levels of xylanase and cellulase concomitantly during SSF while cellulase production was absent in 70% of the strains under LSF (Nair, Sindhu and Shashidhar, 2008). This suggests a higher extra cellular enzyme production and fermentation activity in SSF than LSF method.

Additionally, the hyphal mode of growth of filamentous fungi such as *A. niger* and *T. viride* give them the power to penetrate into solid substrates secreting hydrolytic enzymes at the hyphal tip, without dilution like in the case of LSF (Nair, Sindhu and Shashidhar, 2008). This makes the actions of hydrolytic enzymes very efficient since penetration increases accessibility to nutrients that would result in enhanced nutrient utilization hence increased fungal biomass and crude protein production with time (Raimbault, 1998). This could explain the higher levels of crude protein recorded with SSF method when compared with the LSF method. The crude protein content in the fermented marsh is attributed to extra cellular enzyme production and SCP production.

These results do not agree with those of Guerra *et al.* (1986) who reported that LSF method was more efficient than the SSF method in PW fermentation. A similar conclusion supporting LSF was reported by Stamford *et al.* (1987) using cashew waste as substrate. However, most research work on agro-industrial waste fermentation for SCP
production or chemical production (enzymes, organic acids) have suggested SSF to be superior to LSF and have preferred SSF use in production and experiments to LSF (Penaloza, 1985; Raimbault, 1998; Iyayi and Aderolu, 2004; Perez-Guerra et al., 2003; Iyayi, 2004; Oboh, 2006). The study supports SSF as the preferred fermentation technique for agro-industrial waste fermentation.

The SSF results indicated significant changes in crude protein content of FPW at the three fermentation periods in the order \(48 < 72 < 96\) hours using \(T. \text{viride}\) and \(48 < 96 < 72\) hours in SSF method using \(A. \text{niger}\). This is attributed to the increased hydrolytic enzyme activity with prolonged fermentation and increased fungal biosynthesis resulting in increased biomass hence crude protein, which is an indicator of increased fungal biomass (Raimbault, 1998). A similar trend was not reported with LSF method. This supports the suggestion that the lower hydrolytic enzyme activity in LSF could be due to dilution by the liquid media.

The study reported significantly (\(P < 0.05\)) lower crude fiber in the PW fermented by SSF using both fungi compared to the UFPW for the three fermentation periods. The ability of fungi to degrade crude fiber has been reported by several workers (Iyayi and Aderolu, 2004; Iyayi, 2004). The fermentation process in addition to enriching the substrate with protein also releases oligosaccharides and simple sugars into the medium as a result of microbial degradation of otherwise unavailable polysaccharides (Duru and Uma, 2003; Iyayi, 2004). This suggests the production of hydrolytic enzymes in the fermentation culture by fungi that enable them to metabolize complex carbohydrate polymers (Duru
and Uma, 2003; Iyayi, 2004; Oboh, 2006). This could explain the decrease in crude fiber content of FPW in SSF method.

The study noted that significant decrease in crude fiber was concurrent with significant increase in crude protein especially in SSF method. Other researchers have reported similar findings (Duru and Uma, 2003; Iyayi, 2004; Iyayi and Aderolu, 2004; Oboh, 2006). The carbohydrate content of which crude fiber is a constituent, acts as the carbon source for the growing microbes hence its depletion results from its utilization to produce fungal biomass, which is microbial protein or SCP (Raimbault, 1998).

Crude fiber content was surprisingly high in PW fermented by LSF method using both A. niger and T. viride for the three fermentation periods. The reason for unexpected increase in crude fiber content for the fermented samples may be due to the activities of microorganisms. The fermentation process involves the conversion of materials to the peculiar needs of the microorganisms, which include the bacterial cell wall. The bacterial cell wall is made of peptidoglycan or murein, which is a polysaccharide like cellulose (Eze and Ibe, 2005). As the microorganisms were not separated from the biomass, the increase in fiber could be due to such conversion of materials to peptidoglycan by the microorganisms (Eze and Ibe, 2005). This suggestion coupled with the earlier suggested lower crude fiber degradation in LSF method due to lower extracellular hydrolytic enzyme production and dilution, can be cited as the cause of the higher crude fiber levels in PW fermented by LSF compared with UFPW.
Results for both SSF and LSF method indicate that crude fiber content decreased significantly (P < 0.05) at each fermentation period in the order 48 > 72 > 96 hours using both fungi *A. niger* and *T. viride*. This is attributed to the increased microbial activity with prolonged fermentation and increased hydrolytic enzyme activity that results in increased crude fiber depletion.

Despite the degradation of crude fiber by the fermenting fungi, no significant change in carbohydrate content in PW fermented by SSF method was recorded at 72 and 96 hours using *A. niger* and 48, 72 and 96 hours using *T. viride*, as expected since fungi metabolize complex sugars to simple sugars. This is attributed to the ability of fungi to further hydrolyze the simple sugars for use as a carbon source to synthesize fungal biomass rich in protein (Oboh, 2006).

The increased crude fiber content translated to a significantly (P < 0.05) higher carbohydrate content in PW fermented by LSF method when compared to UFPW. This is so because the carbohydrate content is calculated by summation (Section 2.2.7.6) of which crude fiber is a component. The dilution of the hydrolytic enzymes under the LSF method resulting in lower crude fiber degradation may be the reason for the indiscernible change in the carbohydrate content at the three fermentation periods.

Dry matter content was significantly (P < 0.05) high in all the FPW samples compared to the UFPW. This is in line with the significant (P < 0.05) increase in protein content in all the fermented samples compared to the UFPW. This is attributed to the increased fungal
biomass in the fermented samples because of inoculation and growth of the fungi in the FPW compared to the unfermented samples. The insignificant changes in dry matter levels in FPW at the 48, 72 and 96 hours fermentation periods may be explained by the equal utilization and biosynthesis of nutrient during fermentation hence the lack of significant changes on summation.

There were no discernable trends reported in the specific mineral content (calcium, phosphorous and magnesium) in the FPW compared with the UFPW. Despite this, a significant increase in ash content was reported in all PW fermented by SSF using both fungi when compared to the unfermented wastes. This may suggest the production of specific mineral(s) that were not investigated by this work.

Results of feeding trials using test diets prepared with inclusion of FPW generally indicate that the level of supplementation of FPW in the test diets (50 %) had a negative effect on the performance of the broilers. There was significant (P < 0.05) decline in feed intake and feed conversion by broilers fed on test diets A, B, D and E. This resulted in significantly (P < 0.05) low weight gain (growth) of the broilers, significantly lower than would be expected from the broiler breed used in the feeding trials and in comparison to those fed on the reference diet (Diet C). Where PW was administered 100 %, diets D and E, there was 100 % mortality within the first week.

There are several dietary factors that influence feed intake, especially if the dietary nutrient composition is either deficient or in great excess relative to the broilers
requirements or if the feed produced an illness or other unpleasant post-ingestion effects (Ferket and Gemat, 2006). The low crude protein content of the test diets and the possible presence of unidentified inhibitors of digestive and/or metabolic process in the test diets such as antinutritive factors may have contributed to the poor feed intake, feed conversion and growth response by the broilers. Once feed is consumed, several possible mechanisms regulate further feed intake in broilers. First, they tend to consume feed to satisfy their energy requirements. The second priority is to consume feed to satisfy daily amino acid intake requirements (Ferket and Gemat, 2006). The mechanism that controls feed consumption is sensitive to the concentration of certain amino acids in the blood (Ferket and Gemat, 2006).

Feed intake in broilers is depressed when the protein content of the diet is very low or very high and when the diet is deficient or excess in one or more of the essential amino acids (Waldroup, Jiang and Fritts, 2005). Feed intake is also depressed when the proportions of amino acids in the diet deviate greatly from the proportional amino acid requirements of the organism (Waldroup, Jiang and Fritts, 2005). The levels of supplementation of PW in the formulation of all the test diets resulted in the diets with very low protein content. The values were much lower than the recommended minimum levels for broiler feed of 22 % crude protein. Broilers fed ad libitum with diets having crude protein levels lower than 22 % resulted in a significant reduction in body weight gain and an increase in the feed conversion ratio (Waldroup, Jiang and Fritts, 2005).
Although the study did not establish the amino acid composition of the test diets, it may have been a cause of the poor feed intake by the broilers. Specific amino acid imbalances can modify feed intake in chicks very rapidly inducing growth depression (Waldroup, Jiang and Fritts, 2005; Ferket and Gernat, 2006). Further, specific essential amino acids are necessary for proper growth of broilers. Methionine is considered the first limiting amino acid followed by lysine. Supplementation of the two in crystalline form in poultry diets provides a means to increase the efficiency of protein utilization and reduce the crude protein levels in the diet (Waldroup, Jiang and Fritts, 2005). Guerra et al. (1986) reported that FPW was rich in lysine but was poor in methionine. The crude protein content of the test diets could also have had an imbalance of the essential and non essential amino acids with respect to recommended broiler requirements.

The negative performance of the broilers in this study may have also been as a result of unpleasant post ingestion effect due to the presence of antinutritional factors in the experimental feeds. Post mortem analysis of the broilers that died during the first week of feeding trials as a result of inability to digest diets D and E comprising of 100 % FPW showed that the broilers experienced gut blockage and inability to utilize the feed hence its appearance in the faeces. The unavailable nutrients, poor absorption and utilization of nutrients may have resulted in the death of the chicken through malnutrition.

Presence of FPW in the test diets meant that the feeds had a woody texture much coarser than that of the reference diet (Diet C). The woody texture of the FPW may suggest presence of non starch polysaccharides which pose digestive and metabolic difficulties
for broilers. The non starch polysaccharides cause poor motility of the feed in the broilers gut by increasing the intestinal digesta viscosity which caused increased feed retention time in the gastrointestinal tract of the broilers (Shakouri, Kermanshahi and Mohsenzadeh, 2006). Lower weight gain and higher feed conversion ratio of the broilers on these diets were predictable due to less feed intake and less nutrient utilization because of high viscosity of the chyme and the antinutritional effects of the non starch polysaccharides.

The significantly ($P < 0.05$) high feed conversion ratios of test diets A and B (with PW inclusion) compared with diet C indicated decreased efficiency of feed utilization by the broilers. This is attributed to increased passage of undigested feed in the animals’ gut which is attributed to the presence of antinutritive factors in the diets (Maphosa et al., 2003). Naturally occurring compounds such as protease inhibitors, goitrogens, alkaloids, oxalates and phytates can impair the availability of nutrients, depress feed intake and reduce the growth in animals that consume them (Ferket and Gernat, 2006). Antinutrients which plants such as pineapples use for defense tend to affect the nutritive value of food products by forming complexes with protein (both substrate and enzyme) thereby inhibiting digestion and absorption (Ojokoh and Uze, 2005). PW has been reported to have about 0.09 % phytate. Phytate affects the digestibility of starch by combining with protein in feed or combining with digestive enzymes (Nitithan, Komindr and Nichachotsalid, 2004). This may have contributed digestibility difficulties for the broilers in this study.
Lack of clinical symptoms in the chicken fed on the test diets (containing FPW) suggest that no toxic or pathogenic compounds were present in the FPW or that the residual toxic or pathogenic compounds in the FPW were within tolerable levels.

4.2 Conclusions

i) Fermentation of PW by SSF using the fungi *A. niger* and *T. viride* enriches the nutrient content of the waste, particularly increasing the crude protein and ash content while lowering the crude fiber content.

ii) There are no significant differences (P > 0.05) in the fermentation abilities of the fungi, *A. niger* and *T. viride* with regard to PW fermentation for nutrient enhancement.

iii) SSF method is a more suitable fermentation method over LSF in PW fermentation for sustainable nutrient content enhancement.

iv) Fermentation of pineapple for periods of 72 and 96 hour are equally viable as no significant differences were established when assessing the amount of yield obtained at these two times.

v) The FPW may be a potential supplement in feed formulation due to its non toxicity and non pathogenicity.
4.3 Recommendations

Future fermentation studies using PW as substrate should be planned with the following suggested improvements in the fermentation technique.

i) The SSF fermentation methods outlined in section 2.3.4.3 may be improved by including substrate pretreatment to convert the raw substrate into a more suitable form to increase its utilization by the fermenting microorganism hence better nutrient enhancement. These include size reduction by chopping; Physical chemical or enzymatic hydrolysis of polymers.

ii) Supplementation of the fermentation culture with nutrients (Phosphorous, nitrogen salts) and setting the pH and moisture content using a mineral solution (Guerra et al., 1986).

iii) The level of crude protein and crude fiber in the FPW is adequate to warrant further investigations into its possible inclusion in feed of other livestock such as rabbits and goats.

iv) Investigation of the energy and amino acid balance of the FPW to further establish its suitability as feed supplement especially in poultry.

The feeding trials could be conducted with the following improvements on the methodology.
i) The FPW should be supplemented in the conventional feed in various portions of less than 30% to determine appropriate levels of supplementation in the broiler feed.

ii) The supplementation with FPW should be based on replacing a single component of the broiler meal such as maize or fishmeal content as opposed to a percentage of the whole broiler meal.

iii) More research to characterize the FPW with regard to their digestibility, amino acid profile and content of anti-nutritional factors to guide their inclusion in feeding trials.

iv) Physiological determinations should be conducted during the feeding trials to give a further indication of the effects of supplementation.
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