EFFECT OF RE-FERMENTED BREWERS' DRIED GRAINS ON THE PERFORMANCE OF BROILER CHICKENS

BY

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DECLARATION

I declare that the work in this thesis entitled “Effect of Refermented Brewers Dried Grains on the performance of Broiler Chickens” has been performed by me in the Department of Animal Science under the supervision of professors T.S.B. Tegbe and S. O. Ogundipe.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

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CERTIFICATION

This thesis entitled "Effect of Refermented Brewers Dried Grains on the Performance of Broiler Chickens" by ***NDAMS SAMUEL SHEHU*** meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I solely dedicate this work to my wife (***Mrs. Samaniah S. Ndams***) and children (***Gift and Salome S. Ndams***) who were deprived of my full attention during the entire period of this masters' programme.

ABSTRACT

In a laboratory study, aimed at determining the optimum inoculum concentration and fermentation periods and their effects on nutrients composition of brewers dried grains (BDG), five set of BDG samples were inoculated with rumen liquor (inoculum) at concentrations of 10:1, 10:2, 10:3, 10:4 and 10:5 and subjected to four periods of fermentation (2, 4, 6 and 8 days). Refermentation of all BDG samples resulted in increased percentage crude protein and decreased percentage crude fibre. The BDG sample of 10:2 substrate to inoculum concentration on 2-day fermentation had the highest crude protein content.

The effects of dietary levels of RBDG and 30% RBDG diets supplemented with amino acids and enzyme on performance of broiler chickens were investigated in two feeding trials. In the first nine-week trial, two hundred and forty (240) day old broiler chicks were fed diets containing RBDG at 0, 10, 20 and 30% levels. There were three replications of the four treatments in a completely randomised design. During the starter phase, average daily gain of birds on control diet was similar to those of birds on 10 and 30% RBDG but significantly higher ($P<0.05$) than those on 20%RBDG. Feed intake of birds increased with increased RBDG level across the treatments. Feed to gain ratio for birds on 20 and 30%RBDG diets were similar but significantly higher than

those on 10% and control diets. During the 9-week trial, feed intake was observed to increase across treatment with increased level of RBDG. Birds on the 30% RBDG diet consumed significantly ($P<0.05$) more feed compared to those on the control and the other diets containing RBDG. Weight gain was generally observed to reduce with increase in the level of RBDG in the diets. The efficiency of feed utilization revealed a downward trend as dietary level of RBDG increased. There were no significant differences ($P>0.05$) in dressing percentage, thigh and leg percentages among birds on 0, 10 and 20% RBDG. The gizzard, heart, pancreas and abdominal fat were not affected by the dietary treatments.

In the second feeding trial the effect of supplementing 30% RBDG diet with lysine, methionine or enzyme (Allzyme SSF) was studied. There were six treatments (0% RBDG, 30%RBDG, 30% RBDG plus lysine, 30% RBDG plus methionine, 30% RBDG plus Allzyme at 200g/ton of the diet and 30% RBDG plus lysine, methionine and Allzyme at 200g/ton). During the starter phase (0-4weeks) there was no significant difference ($P<0.05$) in daily weight gain of birds on the RBDG diets when compared with the control. During the finisher phase the diet supplemented with lysine alone or with a combination of lysine, methionine and Allzyme improved daily gain and feed conversion of broilers similar to that obtained with the control diet and significantly higher

(P<0.05) than what obtained with the un-supplemented diet (30% RBDG). Gain of birds on 30%RBDG diet plus lysine, plus methionine and plus enzyme was significantly higher (P<0.05) than those of birds on un-supplemented 30% RBDG diet. Feed intake of birds on all 30% RBDG diets were significantly higher (P<0.05) than those on the control diet. The supplementation of 30% RBDG diets with lysine or methionine or Allzyme or a combination of lysine, methionine and enzyme resulted in improved daily gain and feed utilization. The dressing percentages for birds on all diets were similar. The percentage gizzard was similar between birds on plus lysine, plus enzyme and on a combination of lysine, methionine and enzyme supplemented diets. The percentage abdominal fat between birds on plus lysine and plus methionine diets and between birds on 30% RBDG and plus enzyme diets did not differ significantly. The apparent metabolizable dry matter of the 30% RBDG plus enzyme and diet with lysine, methioine and enzyme combination were similar (P>0.05). The apparent protein metabolism for the control and all supplemented diets (except plus methionine diet) were similar but significantly higher than the unsupplemented 30%RBDG diet. Thus, re-fermentation of BDG with adequate supplementation with amino acids and enzyme improved feed utilization and performance of broilers.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background and statement of the problem

The diet of a particular household in Nigeria today is occasionally supplemented with protein of vegetable sources, which fall short in meeting the protein requirement of an individual. This is as a result of the drastic drop in the daily intake of meat/eggs as a source of protein for an average family. The World Health Organization (WHO) has been working among nations to see that the world problem of malnutrition is solved, particularly in the third world countries. Broiler production has been reported to offer the most rapid and cost effective means of making available, high quality animal protein to man in many parts of the world.

Beszedits and Lugowski as cited by Shier and Purwono (1994) reported an estimated 4 million metric tons annual protein shortfall worldwide. This trend in protein production has left animal producers particularly of poultry, a great challenge to face. Dafwang and Odiba (1993) affirmed that with the national

strategic agricultural postulation for an estimated growth rate of 7% per annum in the production of meat/eggs from poultry and other domestic animals, the animal protein available to the Nigerian would have increased to 5.322g per capita by the year 2010 which is still a far cry from the recommended 34g of animal protein by the Food and Agricultural Organization (FAO). Therefore, the need to strive and meet the FAO recommendation for animal protein intake through commercial broiler production cannot be over emphasized.

Sobamiwa (1998) associated the virtual collapse of the once prosperous poultry industry in Nigeria in recent years to shortage and high cost of feed ingredients. Today the high cost of feed ingredients has been aggravated by the ever-increasing demand for these feed ingredients as both staple foods for man and as industrial raw materials (Sobamiwa, 1998). This has invariably brought about high prices of poultry products. Uchegbu and Udedibie (1998) reported that the demand for conventional feed ingredients in the last few years as industrial raw material and for both human and livestock consumption has pushed its market prices to an alarming height that has directly affected the production cost of farm animals.

Feed millers and livestock producers in recent years are faced with the problem of unavailable and high priced feed ingredients. This phenomenon

has necessitated intensive research into the use of agricultural and agro-industrial by-products for compounding poultry and livestock feeds. The fish and blood meal are feed ingredients of animal origin and together with soya bean meal, groundnut cake; maize etc form the conventional feedstuffs used in the poultry feed industry. Church and Pond (1988) affirmed that all feed ingredients of plant origin with crude protein greater than 20% can be used as protein sources in the diet.

Potential sources of by-products and waste of animal and crops used for compounding livestock and poultry feeds include; animal waste, sea weed and single cell protein (SCP) products such as algae, bacteria and yeast (Church and Pond, 1988). Others include; Cocoa husk (Sobamiwa, 1998), brewers dried grains (Nelson, 1984), sorghum distillers waste (Dairo, 1999), brewers yeast slurry (Ikurior and Akem, 1998), shrimps waste meal (Fanimo *et al*, 1998), wheat offal, rice bran, cassava peals, (Dairo, 1988 as cited by Dairo, 1999).

Among these unconventional feedstuffs, one that readily comes to mind is brewers dried grains (BDG). BDG are residues obtained from the production of beer from barley or other grains solely or in a mixture. BDG is an agro-industrial by-product of the brewing industry and is very much abundant in many parts of Nigeria with low demand.

Brewers dried grains was reported by McDonald *et al*, (1998) to contain about 700-760g water/kg when wet and about 100g water/kg when dry. They reported its energy value as 1710 kcal/kg ME and a crude protein content of 25.3%. They also describe BDG as an excellent source of B-vitamins and trace minerals and a potential substrate for the production of microbial proteins during fermentation. A typical count of bacteria number in the rumen approaches between 25-50 billion/ml. The bacteria dry matter contains about 100g N/kg out of which 80% is in the form of amino acids and the remaining 20% being as nucleic acids (McDonald *et al.*, 1998). The knowledge of rumen fermentation has led to the improvement of plant residues through microbial fermentation.

The disadvantages of using BDG compared to some conventional sources of feed have been identified to include; nucleic acid toxicity, palatability and limitation of sulphur amino acid in the product. The problem of nucleic acid toxicity is said to be solved naturally due to the availability of the enzyme (uricase) in mammals which enables them overcome the problem of nucleic acid toxicity (McDonald *et al.* 1998). The palatability is improved by blending with other feedstuffs and the limitation of sulphur amino acid is overcome by supplementation with synthetic products.

In Nigeria with several brewing industries, BDG is readily available. In Kaduna State, BDG is very much accessible due to the location of two brewing industries (the Nigerian Brewery PLc and International Brewery and Beverage Industries) within Kaduna metropolis.

With this insight and the quest for sourcing cheap feed ingredients, the researcher embarked on this study which was aimed at enhancing the nutritive value of BDG for the monogastrics through fermentation using rumen liquor as inoculum.

1.2 Purpose of the study

The objectives of the study are;

I. To determine the effect on nutrient composition of brewers dried grains when passed through a 2nd stage fermentation process, using rumen liquor as inoculum.

II. To investigate the performance of broiler chickens raised on re-fermented brewers dried grains (RBDG) based diets.

III. To evaluate the performance of broiler chickens raised on RBDG diets supplemented with amino acids (lysine and methionine) and enzyme (Allzyme SSF)

IV. To determine the effect of RBDG diets on carcass characteristics and organ weights of broilers.

V. To determine the digestibility of nutrients by broiler chickens fed RBDG based diets supplemented with amino acids and enzyme

VI. To determine the effect of utilizing RBDG on the economics of broiler production.

1.3 Significance of the study

This study was motivated by the fact that large quantities of brewers dried grains are produced in Nigeria by the brewing industry all year round but they are underutilized in monogastric nutrition. Brewers dried grains utilization could be improved through refermentation. If broiler chickens attain better performance on RBDG, it will be of advantage to the brewing industries, farmers and the government. For the brewing industries, BDG will be in great demand, creating an avenue for profitable disposal of the by-product. For the feed industry and on-farm feed producers, it could be a cheap alternative to the use of high cost conventional feed ingredients. The government has for long banned the importation of conventional feed ingredients in order to encourage sourcing of materials locally with the aim of stimulating local production. By this, foreign exchange will be conserved and this will bring about an increase in the Gross Domestic Product of the nation.

1.4 Research Questions

The research questions for this study are;

1. What are the differences in nutrient composition of BDG when passed through 2nd stage fermentation?
2. What are the differences in performance of broiler chickens raised on the different RBDG diets?
3. What are the differences in performance of broiler chickens fed the different RBDG diets with amino acids and enzyme supplementation?
4. What are the differences in carcass characteristics and organs weight of broiler chickens fed the different levels of RBDG and RBDG diets with amino acids and enzyme supplementation?
5. What are the differences in percentage nutrient metabolism by broiler chickens on RBDG diets supplemented with amino acids and enzyme?
6. What are the differences in cost of feed/kg gain (₦) in broiler production when RBDG diets are fed?

CHAPTER TWO

2.0 REVIEW OF RELETED LITERATURE

2.1 The use of agro-industrial by-products in poultry nutrition

In this decade human consumption of meat and eggs of poultry has increased. This has resulted in increased need for feed ingredients. Thus, there is great need for alternative feedstuffs, which are not competitive with human foods and can be successfully included in poultry diets to replace the commonly used ingredients.

More recently, the growth of animal feed industry has allowed considerable use to be made of by- products. Many of which have some weak points such as: high fibre and low energy contents, high bulk, low density, low availability of amino acids or anti-nutritive factors (Ishibashi and Ohta, 2000). However, the weak points may be overcome by the development of chemical, physical and biological processes, by restricting the amount to be included in diets or by supplementing with the limiting amino acids such as lysine and methionine.

The use of industrial by-products internationally has continued to be of interest to feed manufacturers and nutritionists, and many are being put to use as feed ingredients. The most prominent by-products of plant origin in use

today as enumerated by Bello (1984) are mainly of oil seed mills and brewing industries.

2.2.0 By-products of the brewing industry

In the brewing process according to McDonald *et al*, (1998), barley is soaked first for about six days and allowed to germinate. During this time, a complete enzyme system is developed for the hydrolyzing of starch to dextrin and maltose. This first stage is known as the malting process in which the conversion of starch to maltose and other sugars is said to be complete.

After germination but before mashing, the grain (malt) is dried with proper care not to in-activate the enzymes. The sprouts are removed and are sold as malt culms. The dried malt is crushed and small amount of other cereals (maize or rice) could still be added. Water is sprayed on the mixture and the temperature of the mash increased to about 65^0C. The mashing process provides the enzymes with suitable condition for the conversion of proteins and starch to dextrin, maltose and small amount of other sugars. At the completion of the mashing process, the sugary liquid or 'wort' is drained off, leaving brewers grains as residue, which is sold wet or dried as food for farm animal(s).

The sugary liquid (wort) is boiled with hops, which gives it a characteristic flavor and aroma. The hops are filtered off and after drying are sold as spent hops. The wort is then fermented in an open vessel with yeast for a number of days, during which time most of the sugars are converted to alcohol and carbon dioxide. The yeast is filtered off, dried, and sold as brewers' yeast. The by-products obtained from the brewing process are therefore malt culms, brewer's grains, spent hops and brewers yeast.

The main by-products of brewing industries in Nigeria as reported by Bello (1984) which becomes available for Livestock and poultry feeding are: brewers dried grains and brewers dried yeast. However, with increasing local brewing, another by-product of considerable importance to poultry nutrition is the dried spent sorghum mash which is a residue arising from local brewing of "Burukutu" and "Pito"(Dogari, 1985).

2.2.1 Sorghum beer residue (SBR)

Spent sorghum mash is a by-product of the local breweries. The residue is brownish and odorless when properly dried and has a sour taste. It arises from local brewing of "Burukutu" and "Pito" (Tyokpat, 1989). The local breweries are scattered all over Nigeria especially in parts of Kaduna, Benue, Bauchi, Borno and Gongola States (Dogari, 1985). Inhabitants of the Savannah zone of Nigeria practice the use of the residue for animal feeding, but information

on its nutrient contents and prospects of increased utilization is said to be rather scanty (Tyokpat, 1989).

2.2.2 Brewers' dried grains (BDG)

Bello (1984) and McDonald *et al.*, (1998) defined BDG as dried extracted residue of barley malt, alone or in mixture with other cereals resulting from the manufacture of wort. The fresh brewers grains contain about 700-760g water/kg and may be given to cattle, sheep and horses in this fresh state or preserved as silage alternatively. The wet product could be dried to about 100g water/kg and sold as dried brewer's grains. Brewers' grains are reported to have an exposed storage life of about 4-5 days in warm or hot environment and up to 8days in cold weather or periods (Mowat, 1980).

Lilly *et al.*, (1980) have shown through laboratory and pilot scale studies that wet brewers' grains could be stabilized by incorporating 30% beet molasses and 0.3% potassium sorbate and stored in cooled containers.

Lopez and Pascual (1981) reported that BDG could be produced by oven-drying the wet material at temperature ranges of 80-200^{0}c with minimal losses in the dry matter of the materials. They however, observed that oven-drying the grains beyond 3-6 minutes resulted in significant dry matter losses of 18%. They concluded that temperature of 100-120^{0}c and oven-drying time of 6 minutes had no adverse effects on the nutrient composition of the dried grains.

2.2.3 Nutrient composition of brewers' dried grains.

Uchegbu and Udedibie (1998) reported the proximate composition of BDG (maize/sorghum based) as; 28.25%CP, 13.12%CF, 6.70%EE, 7.36%total Ash, 44.57 NFE and 4.23 kcal/gm of gross energy. Oluokun and Olalokun (1995) reported values of 19.78%CF, 21.65%CP, 7.25%EE, 3.45%Ash, 47.87%NFE and gross energy (kJ/kg Dm of 20700 for proximate composition for BDG).

The nutrient composition of BDG varies from grain source to grain source, and method of brewing (Oluokun and Olalokun, 1995). Bello (1984) showed that BDG contained 18-20%CP, 6.0% oil, 20.0% fibre, 0.2% ca, 0.05% P, 0.4% methionine, 50% TDN and 1960 kcal. ME/kg. Lufadeju (1986) reported the chemical composition of BDG (in g/100g) as; 92.5% dry matter, 3.6 nitrogen, 33.33 acid detergent fibre and 4.6 ash, Almquist (1972) reported that it was fairly rich in essential amino acids, with; 0.9% lysine, 0.4% phenylalanine, 1.0% threonine and 1.6% valine.

Church and Pond (1988) reported the composition of dehydrated BDG as 92%DM, 25.3%CP, 15%CF, 0.29ca, 0.52 p%, and 2080 kcal/kg of ME. They further gave the amino acid composition as; 1.30% arginine, 0.50% histidine, 1.50% Isoleucine, 2.30% leucine, 0.90% lysine, 0.40% methionine, 1.30% phenylalanine, 0.90% threonine, 0.40% tryptophan, 1.20% tyrosine and 1.60% valine.

Perry (1980) reported that although the protein content of BDG was high (about 20-30%) it does not contain a good balance of amino acids. Jensen *et al*, (1976) noted that as a result of the conversion or utilization of the starch in the original cereal grains, low metabolizable energy values are to be associated with the use of BDG in monogastric animals. They further noted that the other nutrient components were concentrated, namely; protein, fibre, vitamin, fatty acids, and minerals

2.2.4 Effect of brewer's dried grains on the performance of chickens

Nelson (1984) reported that 50% replacement of maize with BDG and 5% palm oil supplementation in a broiler starter diet supported satisfactory weight gain, feed consumption and efficiency of feed conversion. A finisher ration containing 25% replacement of maize with BDG plus 5% palm oil supplement supported weight gain, feed consumption and efficiency of feed utilization.

Lopez and Carmona (1981) evaluated BDG at levels of 0, 10, 20, 30 and 40% in broiler diets and reported insignificant differences in average weight gains and feed conversion with up to 20% inclusion level. Feed intake and dressing percentage of birds were also not significantly affected up to this level. They observed that when 20% or more of BDG was used, there were significantly reduced abdominal fat pad and meat tissue with no effects on bone tissue content, while the digestive tract weighed significantly more. Using BDG

from Local breweries utilizing sorghum, maize or millets, White *et al.*, (1979) fed 0, 25 and 40% BDG to laying birds and reported insignificant differences in egg production parameters up to 25% level. At the 40% level however, egg weights and production rates fell drastically and there was an increased rate of feather picking in the flock.

Onwudike (1981) tested BDG at 0, 10, 20, 30 and 40% levels in layer diets. The result indicated that the use of BDG at levels up to 30% resulted in highest feed intake, hen-day production and egg weights. Lopez and Pascual (1981) evaluated spent grains at 0, 15, 30, 45, 60 and 90% levels and indicated that diet with up to 30% BDG were comparable to the control and adequate for laying hens. The 45% BDG diet was acceptable, but there was a loss in body weight and a drop in eggshell quality. The 60 and 90% BDG diets caused a significant drop in egg production, body weight, and egg shell quality and even later led to an inhibition of lay at 90% level.

2.3.0 Nutritive characteristics of sorghum beer residue

Dogari (1985) reported the proximate composition of sorghum beer residue as; 91.0% dry matter, 22.01% crude protein, 4.6% ether extract, 8.40% crude fibre, 0.13% ca, 0.16% P and 8.61% ash. The amino acid profile (g/16gN) was given as; 0.54 Lysine, 0.63 histidine, 0.83 arginine, 1.52 aspartic acid, 0.92 threonine, 1.13 serine, 3.75 glutamic acid, 2.29 proline, 0.77 glycine, 1.90

alanine, 0.49 cystine, 1.27 valine, 0.34 methionine, 1.09 isoleucine, 2.65 leucine, 1.11 tyrosine and 1.46 phenylalanine.

Odiba (1987) reported the nutrient composition of spent sorghum mash as: 22% crude protein, 8.0% crude fibre, and 1760 kcal/kg of metabolisable energy. The nutrient composition of spent sorghum mash before fermentation as reported by Oyegbile (1988) is: 15.7% crude protein, 10% ether extract, 2.0% total ash, and 81.7% carbohydrate. After 7-days fermentation with sour milk it contained the following; 21.6% protein, 24% ether extract, 49.4% carbohydrate and 3.0% ash.

Tyokpat, (1989) evaluated the nutrient composition of re-fermented spent sorghum and reported the following composition; 90.03% dry matter, 34.1% crude protein, 14.26% ether extract 7.20% crude fibre, and 1.64% ash. Abasiekong (unpublished data) presented the proximate values of spent sorghum mash before fermentation as; 92% dry matter, 16.3% crude protein, 2.1% ether extract; and after fermentation as; 88% dry matter, 36.0% crude protein and 7.3% ether extract. Dairo (1999) reported the proximate composition of sorghum distillers waste as; 24.67% CP, 12.64% CF, 4.50 EE, 83.0 DM and 4890.7 kcal/kg of gross energy.

2.3.1 Effect of sorghum beer residue (SBR) on the performance of chickens

Dogari (1985) replaced 10, 20, 30 and 40% of the maize with SBR in diet of 0-5 week old broiler chickens. He reported an insignificant difference in weight gain, feed consumption and feed efficiency when up to 40% of maize was replaced by SBR. At 20% level of maize replacement with SBR in the diet between 6-9 weeks of age, weight gain and feed efficiency were significantly depressed. Tyokpat (1989), observed a significant reduction in feed consumption and weight gain without any significant effect in feed efficiency when 30% of re-fermented spent sorghum mash was incorporated in a broiler finisher diet, replacing about 75% of the protein source.

Dogari (1985) observed that age of the bird is an important factor in the practical consideration of the use of beer residue when weight gains are considered. He suggested that it is practicable to replace up to 50% of the maize in the diet of young chicks of between 0-5 weeks and 60% of maize in the diet of older birds (6-9 weeks). Where energy may be a limiting factor, levels of replacement of maize not exceeding 40% were suggested as safe.

2.4.0 Fibre in monogastric nutrition

The digestibility of a feed ingredient is closely related to its chemical composition. The fibre fraction of a feed has the greatest influence on its

digestibility, and both the amount and chemical composition of the fibre are important (McDonald *et al.* 1998).

High fibre basal diets according to Yaakugh (1986) are normally fluffy and carry a low proportion of nutrients per unit volume rather than per unit weight. He affirmed that substituting high fibre feeds (on weight for weight basis) would generally result in reduced nutrient intake by animals and higher fibre levels in the diets. Industrial by-products of plant origin (especially those of milling and brewing industries) are marked with a high level of crude fibre, which limits their use in monogastric nutrition. In ruminant animals, the large microbial population residing in the rumen contains enzymes capable of hydrolyzing the fibre materials present in the cell walls, particularly those associated with the leaf and stem portion of the plant and outer bran layer of seeds. Non-ruminant animals (horse, pigs and human) utilize some of these fibrous feeds to a small extent by virtue of the presence of similar microbial population residing in the colon and cecum (MacDonald et al., 1998). Ruminants consuming all forage diets obtain most of their energy as volatile fatty acids (VFA) produced by anaerobic microbial fermentation of fibre in the rumen (Church and Pond, 1988).

Maisamari (1986) asserted that in the feeding of farm animals, it is important to know the fibre composition of various feeds since fibre of different origin is

often digested differently and their nutritional value varies for different animal species.

2.4.1 The effect of fibre in pig nutrition

In feeding trials with pigs, Delic *et al.,* (1977) evaluated BDG as a protein source where it constituted 10% of the diet and observed a better feed consumption per unit live weight gain. Improved live weight gains were recorded when lysine was added to the diets. More muscles were deposited per kilogram weight gain. The improved gains on lysine supplementation revealed lysine inadequacy in BDG as a feed ingredient.

Kornegay (1973) fed BDG at 0, 25, and 50% levels to growing pigs and discovered an insignificant difference in feed requirement per unit gain. At 50% level, the average daily feed intake was depressed. He observed no significant difference in average daily gains. Buthia *et al.*, (1975) fed growing pigs with 0, 40, 50, and 60% levels of BDG and reported that up to 40% inclusion rate depressed gains with longer time to attain 60kg weight. In finishing pigs, Palev *et al.*, (1982) after feeding 0, 5, and 10% of BDG in diets observed that BDG inclusion brought about higher feed intake and depressed weight gain.

Dairo (1999) evaluated the effect of sorghum distillers waste (SDW) at 0, 10, 15% and 20% levels of inclusion on the performance of pigs and observed the

average daily feed consumption to be influenced significantly. It was observed that weaned pigs on 15% SDW had the highest feed intake, while those on 0% SDW had the lowest feed intake. Similarly, Lindervan *et al.,* (1986 as cited by Dairo 1999) observed that at growing and finishing phases, pigs on 0 and 10% SDW had the highest feed consumption. Higher inclusion levels of 10, 20, and 30% SDW resulted in marked decrease in feed intake.

Research has also shown that higher levels of dietary fibre in pigs depress growth and increases feed intake significantly. Frank *et al.,* (1984) fed 0, 7.5 and 15% corn cobs to growing pigs and reported linear decrease in average daily weight gain (ADG) without any significant effect on average digestible energy intake.

2.4.2 Effect of fibre in poultry nutrition

Studies have been carried out to investigate various effects of fibre in poultry nutrition. Church and Pond (1988) have established the favorable effects of fibre inclusion in poultry diets. They stressed the need to incorporate fibre in concentrate mixtures to attain an amount of bulk in order to avoid the formation of dough like mass in the stomach, which is not readily attacked by digestive enzymes. For the unfavorable effects of fibre in the diet, the authors revealed that fibrous feeds might decrease the digestibility of crude protein

and ether extract. They claimed that increased bulkiness of the ration consequent to the inclusion of fodder might lead to a more imperfect contact between proteolytic enzymes and the basal feed. There is further possibility that the presence of the fibre may speed up the rate of passage of food through the simple stomach of monogastrics. The authors further stated that fibrous feeds may increase the output of nitrogenous material into the feaces thus causing a depression of the apparent digestibility and increased nitrogen excretion through ionic interaction matrix restriction and modification of filtration characteristics of the fibre components.

McDonald *et al.* (1998) stated that the crop by virtue of its physical similarity to the rumen of ruminants may have the potential for digestion of lower quality fibrous diets that are presently being fed to poultry. It was concluded that fibre aids digestion since it stimulates peristalsis and secretion as well as opening up concentrate feeds and bringing them into contact with digestive juices. Feed consumption and body weight gains especially in meat type birds decreased when birds were fed high fibre diets, (Ndams, 1991).

Nnaemeka (1980) revealed why high fibrous diets are less used in monogastric nutrition and how their digestibility must be improved through various treatments if they are to be applied in poultry nutrition in particular.

2.5.0 Improvement of the nutritive value of feedstuff through various treatments

A wide variety of agro-industrial by-products have been fed to farm animals with varying degree of success. According to Walker, (1984) and Macdonald *et al*. (1998) the low protein content and poor digestibility due to high cellulose content of agro-industrial by-products have often given poor results when they are fed to uphold high level of meat, eggs or milk production. The commonest treatments applied to feedstuffs as enumerated by McDonald *et al.* (1998) include; chopping, chaffing, crushing or grinding and cooking.

Church and Pond (1988) stressed that it is imperative to supply an adequate diet in a manner that will encourage consumption without waste and allow high efficiency of utilization. Alterations of feed ingredients before feeding to animals have been reported to be accomplished through physical, thermal, biological and chemical methods. Church and Pond (1988) enumerated the effect of feed processing to include; alteration in physical form or particle size, preservation, isolation of specific parts, improvement of palatability or digestibility, to alter nutrient composition or for detoxification.

According to Tyokpat (1989) the different methods of feed treatments can be classified into physical, chemical and biological.

2.5.1 Physical treatments

Physical treatment methods, which have been used to increase the digestibility of low quality feedstuffs, include; grinding, ionizing radiation and steaming.

2.5.1.1 Grinding

The reduction of particle size by grinding has been effective in improving the nutritive values of ligno cellulosic residues. Mechanical crushing of coarse agricultural residues has been known to increase daily intake of animals, probably because of increase in the density of the feeds and reduction in chewing time (Walker, 1984). The grinding of roughages therefore increases the digestibility of their crude fibre by as much as 20 percentage unit (Macdonald *et al*.1998).

2.5.1.2 Ionizing radiation

The digestibility of ligno-cellulosic materials is increased with the use of ionizing radiation (a process known as micronization). In micronizing, heat is provided in the form of infrared energy. When lignocellulosic materials are irradiated, cellulose chain length decrease and insoluble carbohydrate component becomes more available (Church and Pond, 1988).

2.5.1.3 Steam treatment

This is a form of heat treatment of feedstuffs to improve their digestibility. Heat is directed at the feed material in form of steam. Heat treatments are reported (McDonald *et al*, 1998) to be most effective in improving digestibility when used for the specific purpose of inactivating the digestive enzyme inhibitors that are present in some feeds (e.g. protein concentrates of plant origin).

The preferred method of treating ligno-cellulosic plant materials now according to Walker (1984) is the high-pressure steam method. He observed that steam treatment processes cost much due to high energy that is involved.

2.5.2 Chemical treatments

Chemical treatment enhances accessibility of carbohydrate in lignocellulosic materials. Chemical treatment as reported by Fanta *et al*, (1984) involved removal of hemicellulose and some lignin leaving pores large enough to permit enzymes access to the carbohydrate fractions.

When straw is exposed to an alkali, the ester linkages between lignin and the cellwall polysaccharides, cellulose and hemicelluloses are hydrolyzed, thereby causing the carbohydrates to become more available to the micro organisms in the rumen of ruminants (Church and Pond 1988).

McDonald *et al.*, (1998) observed that chemical treatment of straws have attracted a great deal of research interest but their use in practice is somewhat limited due to the fact that countries to benefit most have neither sufficient resources of chemicals nor the technology needed to apply them. Chemical treatment methods of straw ranges from soaking straws, spraying, ensiling with alkali and treatment with anhydrous ammonia (Church and Pond, 1988). Sobamiwa and Longe (2003) investigated the effect of alkali concentration and treatment on the chemical composition of cocoa pod husk (CPH) and reported decreased amount of crude fibre, hemicellulose, cellulose and CP but increased total ash, Na, Cu, Fe, Mn, and Zn contents of CPH. The incorporation of the alkali treated CPH (TCPH) in broiler starter diets at levels of 0, 50 100 and 150g/kg did not significantly reduce growth performance and apparent digestibility at levels up to 100g/kg TCPH inclusion at 150g/kg was observed to significantly reduce weight gain and feed efficiency compared with those on the control diet.

2.5.2.1 Soaking

The Beckman soaking method (Homb, 1984 and Mc Donald *et al*, 1998) requires two barrels. One for soaking in NaoH solution, and the other for washing in water. In the process, straw is soaked for 1-2days in a dilute solution (15-30g/l) of sodium hydroxide, then washed exhaustively to remove

excess alkali. The process increased dry matter digestibility from 0.4 to 0.5-0.7.

2.5.2.2 Spraying

In this method, chopped or milled straw is sprayed in a mixer with a small volume of concentrated sodium hydroxide. McDonald *et al.,* (1998) recommended that 170 l/t of straw for a solution of 300g/l of NaOH will supply 50kg NaOH. This process is believed to improve digestibility to a slightly lesser extent than the Beckman soaking method. Refugio and Dolores (1993) determined the effect of spraying chemical solutions (NaOH, $Ca(OH)_2$ NH_4OH and H_2O_2) on sugar cane bagasses on single cell protein production. They observed decreased lignin content as a function of time during pretreatment with calcium and sodium hydroxide showing low delignification and relatively high amounts of phenolic compound released. They also observed that lignocellulosic residues treated by spraying NaOH and $Ca(OH)_2$ solutions on pith resulted in increased cell growth and biodegradation of pith compared with NH_4OH and H_2O_2 pretreatment.

2.5.2.3 Ensiling

Ensilage implies preservation by bacteria fermentation of carbohydrate to short chain inorganic acids such as lactic and acetic acid (Wilkinson, 1984).

Wilkinson (1984) observed physical changes associated with the ensiled materials after treatment with NaOH to include: heat production, loss of structural rigidity and color change to bright or deep yellow or yellow/brown. Sundstol *et al.,* (1979) reported that when relatively raw straw was treated with only 0.1 litre of NaOH solution per kg straw, and ensiled, there was progressive, increase in digestibility during 63-days storage period.

Chaudhry *et al.,* (1998) investigated the effect of ensiling broiler litter on chemical composition and pathogenic organisms. They ensiled broiler litter at 40% moisture with rumen contents in 40:60 and 50:50 ratios, wet basis for 42 days. They achieved desirable fermentation in all the silage, with significant reduction in pH and water-soluble carbohydrates, and significant increase in lactic acid.

Mthiyane *et al.*,(2001) investigated changes in chemical composition, fermentation characteristics and mycotoxin-producing fungi following ensiling of sugarcane tops with broiler litter with and without water for 45days. It was observed on the basis of chemical analysis that ensiling with broiler litter and water decreased the CP and GE contents whilst with broiler litter alone increased the DM, CP, and ash, Ca, and P contents of silages.It was further observed that ensiling destroyed Fusarium and Rhizopus and reduced the incidence of scopulariopsis fungi while Aspergillus,

Scopulariopsis and Penicillium fungi showed the ability to survive under anaerobic conditions.

2.5.2.4 Ammonia treatment

Anhydrous and aqueous ammonia act as delignifying agent on straw (Sundstol, 1981). Chomyszyn *et al.*, (1960) soaked chopped straw in an ammonia solution mixed with hay and field peas and stored for 3-days. It was concluded that ammoniation of straw improved palatability of the ration, improved digestibility and increased the rate of gains and the efficiency of feed utilization in ruminants. The danger observed from ammonia treatment was that it might sometimes cause the production of toxic imidazoles, which arise from reactions between ammonia and sugars.

Urea has been reported as a source of ammonia for hydrolysis. Thus KNARDA **(**1987) recommended that 1kg of fertilizer grade urea be dissolved in 10 liters of water and sprinkled on 20kg straw in a pit and covered for 20days before feeding to ruminants. Animal urine, which is abundant in livestock farms, has been reported as a source of ammonia. Sundstol (1981) reported that the use of animal urine at the rate of one litre per kg straw stoked above ground for 20days, increased organic matter digestibility from 45 to 55%.

2.5.3 Biological treatment

Zandrazil (1984) pointed out that the main problem of biological upgrading of ligno cellulose materials into feed as being the availability of suitable microorganism with metabolic pattern different from those of the rumen flora and fauna. He reported that sweet rot fungi, similar to bacteria and yeast only metabolize soluble sugar or other easily digestible cell constituents of plant residue while more resistant plant polymers (cellulose and hemicelluloses) are primarily decomposed by the brown rot fungi at extremely low pH (3.0-2.0).

2.6.0 Rumen microbiology

Ruminant nutritionist and microbiologist have long been interested in manipulating the microbial ecosystem of the rumen to enhance feedstuff utilization, improve production efficiency by ruminants and alleviate problems associated with current feeding practices (Lee, *et al.*, 2000).

The reticulo-rumen provides a very favorable environment for microbial survival and activity. According to Church and Pond (1988), a typical count of bacteria number in the rumen approaches between 25-50 billion/ml and typical values of protozoa counts expected are in the order of 20,000-50,000/ml. Yeast was also reported to occur sometimes in large number but not with great regularity. McDonald *et al*., (1998) reported that rumen content contains 850-930g water/kg on the average and the number of bacteria per ml of rumen

content between 10^9- 10^{10} with over 60 species identified. Protozoa was observed to be smaller in number (10^6per ml) than bacteria. The bacteria dry matter contains about 100g N/kg out of which 80% is in the form of amino acid and the remaining 20% being present as nucleic acid (McDonald *et al.*, 1998).

Feed and water enter the rumen and is partially fermented to yield principal volatile fatty acids, microbial cells and the gases (methane and carbon dioxide). Greater part (sometimes all) of the proteins reaching the small intestine is microbial proteins also called single cell proteins (Church and Pond 1988).

Microorganism according to Tegbe (1976), have rapid growth rates and their growth is not affected by climatic fluctuations. He affirmed that single cell protein (SCP) can be produced in large amount without climatic disturbances.

The disadvantages of using SCP compared to conventional sources of proteins were identified to include; the toxicity of nucleic acid to humans, the non palatability of the SCP causes low feed intake and the limitations of the sulfur amino acids in the product (Tegbe 1976). The problems listed above however are not without solutions. According to Tegbe (1976) the availability of the enzyme Uricase in the mammals enable them overcome nucleic acid metabolism. The acceptability of SCP has been improved through blending

with other feedstuffs and the limitation of sulfur amino acids has been overcome with synthetic methionine supplementation.

Lignocellulose materials that have been studied as substrate for the production of SCP according to Joao *et al.,* (1995) include; rice straw, corncobs, sugar bagasse and eucalyptus. By-product feeds that are low in rumen available carbohydrate may reduce microbial protein output (Stokes *et al.*, 1991 b). Brewers' grains are by-product of the brewing industry consisting primarily of the extract of residues of the grains used in the brewing industry (Merchem *et al.*, 1979). Church and Pond, (1988) reported an energy value of 2080kcal/kg and a crude protein content of 25.3% for BDG. This makes it a potential substrate for the production of microbial proteins (SCP) during fermentation.

2.7.0 Effect of fermentation on nutrient composition of feedstuffs

The knowledge of rumen fermentation has led to the improvement of the nutritive value of plant residues. Stewart (1981) affirmed that an "ideal" fermenter digesting lignin and cellulose containing plant residues could be found in the rumen of both domestic and wild animals. Pido *et al.,* (1979) demonstrated detoxification of cassava products by fermentation, while Osuji (1982) reported that farmers in Grenada ensiled citrus pulp in unused artisan

well for dry season feeding and such Silage were relished by both sheep and cattle.

Ofuya and Obilor (1992) fermented fresh cassava peel for 4-days (96hrs) at 30^0c using Rhizopus sp. as inoculum. They reported the proximate composition of cassava peel before fermentation as; 28% DM, 5.4% CP, 1.2% EE, 20.7% CF, and 5.8% Ash and after fermentation as; 90% DM, 16.9% CP, 3.0% EE, 24.3% CF and 4.9% Ash. The fermented cassava peel when fed to young poults in starter diet was more acceptable with better growth than birds fed on unfermented diet.

Kanazas and Field (1981) reported improved protein quality from 70% to 83.7% with increased available lysine, leucine, methionine and reducing sugars for fermented Sorghum. Oyegbile (1988) carried out a 7-day fermentation of spent sorghum mash (SSM) and observed crude protein and increased ether extract. Joao et *al.,* (1995) fermented Eucalyptus hemi cellulose hydrolysate to produce microbial protein by Paecilomyces variotii (reported to be the first fungus to be utilized in an industrial process for the production of microbial protein). The protein was reported to contain all the essential amino acids for animal feeds. Agro-industrial by-products that have been studied as substrates for the production of microbial protein include;

sugar bagasses, rice straw, corncob and Eucalyptus (Almaida e Silva *et al;* 1995 as cited by Joao *et al.,* 1995).

2.8.0 Amino acids supplementation

The nutritive value of proteins varies between feedstuffs. Studies have indicated that the requirement for the essential amino acids is a function of the total protein content of the diet (Sklau and Plarnk 2002). The amino acid quality of a feedstuff determines the extent to which its protein is utilized.

According to Ishibashi and Ohta (1994), it is not protein but amino acids, which is needed for the maximum weight gain in broilers. While the weak points in these feed ingredients are usually overcome through physical and chemical processes, and restriction of the amount of the ingredient to be included in the diet. The low availability of amino acids has been overcome by supplying the limiting amino acids such as lysine and methionine. Ishibashi and Ohta (1994) established that when single or multiple amino acids are deficient, feed intake and performances of animals are low. The performance of animals is recovered by addition of deficient amino acids. Hikimi *et al*., (1988) reported that when methionine deficient diet was supplied, growth and feed intake of some broiler chicks was depressed. It has been reported that when the dietary amino acid is lower than the requirement, young chicks

refused to eat the diet to meet the ME requirement. However, older chickens tend to eat the diet until the deficient amino acid is satisfied (Sugahara *et al.,* 1995).

Babatunde and Fetuga (1976) found that when there is an imbalance in the amino acid profile, the optimal performance of the broiler is not attained. According to Lemme and Petri (2002), carcass evaluation in turkey birds at 20 weeks of age showed significant increased in breast meat yield with increasing dietary lysine levels. The proportion of the drumsticks and wings were not affected and the proportion of thigh tends to decrease with higher dietary lysine content.

Wyclif *et al.,* (2003) evaluated the effect of dietary protein concentration and specific amino acids on body weight, body composition and feather growth in young turkey. They observed increased body and muscle weight on amino acid supplementation. Birds on tyrosine supplemented diets were observed to have reduced featherweight, whereas valine had no effect when compared to the basal ration. Supplementation with arginine and methionine resulted in increased featherweights that were similar to that of the control.

2.9.0 Enzyme supplementation

Enzymes are biological catalysts for organic reactions. They play key role in the digestive processes of the animal. They are protein containers by nature

and are produced by the animal itself or by the microbes that are naturally present in the digestive tracts. High molecular nutrients have to be digested to small size by digestive enzymes in the gastro intestinal tract. However, monogastrics (e.g. Poultry) have none or few of such enzymes that can digest nutrients like; cellulose, phytate and some proteins (Ishibashi and Ohta, 2000). Phytate makes complexes with proteins and inhibit a number of digestive enzymes such as pepsin, alpha amylase and trypsin. This inhibition may lead to low digestibility of proteins and energy. The addition of β-glucanase and xylase to barley and wheat based diets improved performance of broilers and efficiency of dietary nutrients (Ishibashi and Ohta,1994). The supplementation of cellulase and protease may increase the utilization of cellulose and proteins respectively (Ishibashi and Ohta, 1994).

Iji *et al.* (2001) supplemented a wheat-based diet with a microbial enzyme, Avizyme 1300 (FinFeeds International UK) which has predominantly xylanase (2500 iu/g) and protease (800 iu/g) activities. They observed improvement in the dietary apparent metabolisable energy content with slight but insignificant positive effects on chick growth and feed conversion ratio. Choct *et al.*, (1994) reported the enhancement of non-starch polysaccharides digestion by microbial enzymes leading to improvement in animal performance.

Wu *et al.*, (2003) evaluated the effect of microbial phytase *Apergillus niger* (Allzyme SSF; Alltech Inc. Nicholasville KY) produced by solid state fermentation, on the performance and nutrient utilization of broilers fed maize and wheat based diets. They observed improved weight gains irrespective of diet type and an improved apparent metabolizable energy values of wheat-based diets and little effect on apparent ME of maize-based diets. The enzyme (phytase) improved ileal nitrogen digestibility in both maize and wheat based diets but the response to added phytase tended to be higher in wheat based diets. This enzyme product was guaranteed to contain a minimum phytase activity of 1000 phytase units per gram. The phytase product also contained several side enzyme activities including; protease, amylase, cellulase, xylanase and β-glucanase (Wu *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1.0 Nature and location of the experiments

The experiments were conducted in two phases. The first phase consisted of laboratory studies. This involved the determination of the proximate and amino acid composition of refermented brewers dried grains (RBDG) as well as microbial analysis of the wet RBDG. The proximate analysis was carried out at the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria, according to A.O.A.C (1990). The samples were analysed for amino acid composition at the department of Zoology, University of Jos, Nigeria. The microbial analysis was carried out at the Medical Laboratory, Yusuf Dantsoho Hospital, Tudun Wada, Kaduna.

The second phase of the experiment consisted of feeding trials. The growth performance and carcass characteristic of broiler chickens fed diets containing RBDG were evaluated. The metabolism of nutrients in RBDG diets supplemented with amino acids and enzyme was also evaluated. The feeding trials were conducted at the Poultry Production Unit, Government Girls Secondary School, Kawo, Kaduna.

SECTION A

3.2.0 Sources and nature of brewers dried grains (BDG)

BDG used in this trial was sorghum based. It was characteristically brownish in colour and appears fibrous. Dried samples are odourless with a sour taste. All samples used in the laboratory studies and in experiment I and II were sourced from middlemen at Nasarawa village, Kaduna who purchase wet spent grains from the International Beer and Beverage Industries (IBBI) makers of Kronengburg Lager beer located within Kaduna metropolis. The middlemen usually dry the spent grains for between 2-3 days to produce the brewers dried grains.

3.3.0 Determination of the optimum inoculums concentration and fermentation period and their effect on nutrient composition of brewers dried grains.

The objectives of this study were: -

1. To determine the best inoculums (rumen liquor) concentration for BDG fermentation.
2. To determine the optimum fermentation period (days) for BDG re-fermentation.
3. To determine the nutrient composition of BDG when subjected to a second stage fermentation (re-fermentation) process.

3.3.1 Inoculum preparation

Rumen content of a cow was collected fresh just after slaughter at Kawo abattoir, Kaduna. This was then mixed with water in a 2:1 ratio (w/w) and filtered through a mosquito net of pore size 2.78mm^2 (Tyokpat, 1989). The residue was discarded and the filtrate used as inoculum**.** The method of inoculum preparation for BDG inoculation and fermentation in experiment I and II was similar to what was done here.

Two test tubes containing 5mls of the filtrate (inoculum) were preserved in a refrigerator for laboratory identification of the type of bacteria present in the rumen liquor (inoculum).

3.3.2 Inoculation:

Five (5) different concentrations of substrate to inoculum ratios were used (10:1, 10:2, 10:3, 10:4 and 10:5, w/w. The 5 different inoculated samples were each subjected to four periods of fermentation. Twenty-one (21) bottles (empty jam containers with covers including one for uninoculated sample) of equal size were used. Each BDG inoculated sample (10:1, 10:2, 10:3, 10:4, and 10:5) was subjected to 4 different periods of fermentation (2, 4, 6 and 8 days). Five (5) bottles were used to carry the same inoculated BDG sample (i.e. a set for each of the samples' concentration of 10:1, 10:2, 10:3, 10:4 and

10:5). Each set was then subjected to 4-different periods of fermentation (2, 4, 6 and 8 days). The quantity of water that will wet a given quantity of dried BDG was first determined. This was found to be in a ratio of 5:1 (w/w). The *5* different inoculum concentrations (10:1, 10:2, 10:3, 10:4 and 10:5) were first introduced into the water before wetting the BDG samples for uniformity of inoculation.

Quantitatively, 5 buckets were kept and 3kg of BDG were weighed into them. The following weights of inoculum (rumen liquor); 0.3kg, 0.6kg, 0.9kg, 1 .2kg and 1.5kg corresponding to the substrate to inoculum ratio of

10:1, 10:2. 10:3, 10:4 and 10:5 were weighed and introduced into equal volume of water. Properly mixed and each volume of water containing the different concentration of inoculum was used to wet the 3kg of BDG in the bucket in a ratio of 1:5 (w/w). The different ratios used are presented in Table 3.0.

Table 3.0: The ratio of substrate to water and to inoculum concentration.

Sample			Fermentation periods (days)			
Label	[1]S:[2]W	S:[3]I	2	4	6	8
A	1:5	10:1	A2	A4	A6	A8
B	1:5	10:2	B2	C4	B6	B8
C	1:5	10:3	C2	C4	C6	C8
D	1:5	10:4	D2	D4	D6	D8
E	1:5	10:5	E2	E4	E6	E8

[1]substrate [2]water [3] inoculum

Four of the bottles were allocated to each of the five different BDG inoculated samples (totaling 20 bottles) to which 0.5kg of the BDG inoculated samples were filled. The four bottles from each bucket were then subjected to four different fermentation periods (2, 4, 6 and 8 days).

3.3.3 Fermentation

The inoculated BDG samples were properly compacted into the four-bottle (500g) to exclude any possible air. The mouth of the bottles were covered with polythene materials and finally sealed with covers and buried under the soil so as to maintain anaerobic condition as much as possible.

All bottles of the inoculated BDG samples were labeled accordingly and fermented for between 2 to 8 days. Another bottle with wetted BDG material was fermented for 8 days un-inoculated, making a total number of twenty-one (21) bottles used in this study.

After each period of fermentation, 10 gm of the re-fermented BDG sample was taken into text tubes. The twenty (20) test tubes containing wet re-fermented BDG material were properly labeled and kept in a refrigerator for bacteria count which was carried out at the medical laboratory, Yusuf Dantsoho Hospital, Tudun Wada, Kaduna.

The rest of the re-fermented BDG (21 samples) were removed from the bottles and sun-dried for 3 days after which samples were taken for chemical

analysis. The proximate chemical determination for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash and nitrogen free extract (NFE) were carried out at the National Animal Production Research Institute (NAPRI), Shika- Zaria, Kaduna State. The results of the proximate analysis and bacteria population determined were as shown in tables 4.2 and 4.2.

3.4.0 Chemical analysis

The analytical procedures (for DM, CF, CP, EE, Ash and NFE determination) employed in this experiment are in accordance with methods of AOAC (1990). Proximate analysis was carried out on the BDG sample and the 21 RBDG samples. for dry matter (DM), ash, ether extract (EE), crude fibre (CF), nitrogen (N_2) and crude protein (CP) determination.

3.5.0 Microbiological analysis

The 5-ml rumen liquor that was preserved in the refrigerator was taken for the identification of the type of bacteria present in it at the laboratory. Likewise the twenty wet refermented BDG samples of the five different inoculum to substrate concentration were taken for bacteria count. This was to identify the substrate to inoculum concentration that will result in the highest bacteria count. The procedure was as follows:

3.5.1 Media preparation

12.4g of nutrient agar (bacteria growth media) was added to 450 ml of distil

water and mixed. The media contained in a beaker was then sterilized by autoclaving at 121^0c for 15 minutes. The media was then cooled in water bath at between 25 ^{0}c to 45 ^{0}c before it was poured on the plates.

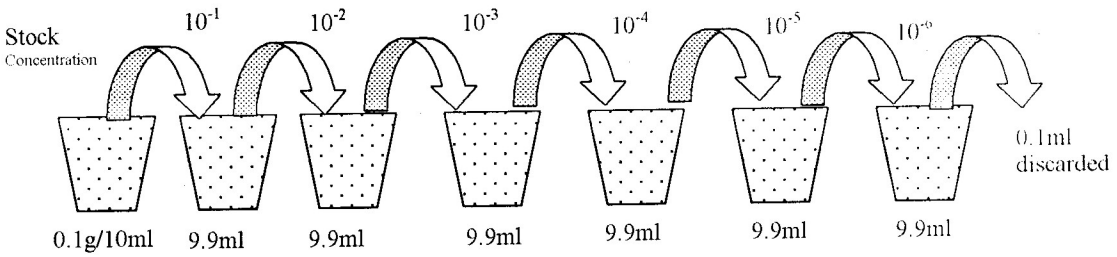
3.5.2 Preparation of the dilution

1 gm of each wet RBDG sample was weighed and mixed in 100ml sterile distil water and out of this, 10 ml, were taken in a test tube as the stock bacteria concentration. This contained 0.lg of the RBDG sample (0.1g/10ml).

3.5.3 Serial dilution

To reduce the bacteria concentration before inoculation of every sample, six (6) screw cap test tubes were kept, into which 9.9 mls of 0.1% peptone were put to serve as dilution blank. The test tubes were arranged in series from 1 to 6. From the stock concentration, 0.l ml was removed and transferred into the first dilution blank as shown in figure 2.0. These were mixed and from it 0.1ml was removed and discarded every time to allow equal concentration.

Fig 2.0



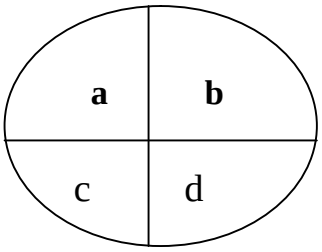
3.5.4 Inoculation of plates

The 5th and 6^{th} dilutions were used for plate inoculation. In the process, I ml of each dilution (10^5 and 10^6) was removed with a pipette and introduced into the center of each sterile petri-dish. 10ml of nutrient agar (cooled to about 45 ^{0}c) were poured on each plate and mixed by moving the dish gently, six times in clockwise direction and repeated counter clockwise. After the media was allowed to set for 20 minutes the plastic dishes were inverted and incubated at 37 ^{0}c for 24hrs.

3.5.5 Bacteria count on plates

The plates were divided into four quarters as shown in figure 2.1. Each quarter was counted and the count of the four quarters added to obtain the total count.

Fig.2



Count per plate = a + b + c + d. The results of the bacteria count are as shown in table 4.6.

3.5.6 Gram staining

The 5-ml rumen liquor preserved for bacteria identification was used to inoculate a plate of MacConkey agar. From the colonies of bacteria growth a

smear from it was made on clean slide. Also a smear was made from the bacteria colony grown on nutrient agar for population count. The smears were dried and fixed by passing it briefly through a bunsen flame. The smears were covered with crystal violet stain for 30-seconds and washed. They were covered with Lugol's iodine for 30-seconds and again washed clean with water. Rapidly, the smear was decolorized with acetone followed by clean water washing. The smears were again covered with neutral red for one minute and again washed with clean water. The slides were kept on a rack to dry after cleaning the back with absorbent paper.

3.6.0 On-farm preparation of refermented brewers' dried grains (RBDG)

3.6.1 Inoculation and fermentation

The inoculation of BDG prior to fermentation was carried out as described in the laboratory study. Four bags (50kg each) of BDG were inoculated on a concrete floor and then turned into a 5-feet deep pit lined with polythene sheets. It was properly compacted by pressing under feet and covered with polythene, soil and zinc sheets to maintain as much anaerobic condition as possible. The substrate (BDG) was allowed to ferment for 2- days. At the end of the fermentation, the pit was opened and observation was made on the colour, odour and consistency of contents. The re-fermented BDG was then

sun dried for 3 days after which samples were taken for chemical analysis according to AOAC (1990) procedures. The results of the chemical analysis determined are as shown in table 4.7.

3.6.2 Determination of amino acid composition

The amino acid profile in the RBDG sample was determined using methods described by Sparkman *et al*,. (1958). The results of each amino acid concentration obtained by calculation were as shown in table 3.2.

Table 3.2: Amino acid concentration (g/100g protein) of RBDG

Amino acid	Concentration (g/100g protein)
Lysine	1.48
Histidine	1.80
Arginine	2.95
Aspartic acid	5.08
Threonine	2.04
Serine	1.76
Glutamic acid	12.48
Proline	6.16
Glycine	1.84
Alanine	6.14
Cystine	1.61
Valine	4.49
Methionine	1.12
Isoleucine	3.10
Leucine	8.12
Norleucine	-
Tyrosine	3.04
Phenylalanine	4.02

SECTION B

FEEDING TRIALS

3.7.0 Experiment 1. Effect of feeding graded 1eve1s of RBDG on the performance and carcass characteristics of broiler chickens

The performance of broilers was evaluated in a 9-week trial where RBDG was fed at graded levels (0, 10, 20, and 30%) during the starter and finisher phases. The ingredient composition of the broiler starter and finisher diets are as shown in tables 3.3 and 3.4, respectively.

3.7.1 Broiler starter phase (0- 4 weeks)

Two hundred and forty day-old broiler chicks were randomly allocated to 12 pens in groups of 20 birds per pen. Four diets were prepared in which RBDG was fed at graded levels of 0, 10, 20 and 30%. Each diet was replicated three times. The diets were randomly allocated to the pens of broilers with concrete flooring; each pen measuring 3.5 by 7.0 m. Wood shaving was used as litter materials.

All the test rations were iso-nitrogenous but not iso-caloric. The starter diets were formulated to have 23% CP. The respective groups of chickens were fed the starter diets (Table 3.3) for 4 weeks. All birds were provided with feed and water *ad libitum.* Weighed quantity of feed was given daily and leftover feeds were collected and weighed. The birds were weighed at the

commencement of the experiment and subsequently at weekly interval. The record of feed consumption was also kept. Electrical power supply was very poor during the period of the study and therefore lighting was most often natural. Record of mortality was kept throughout the period.

3.7.2 Broiler finisher phase (5-9 weeks)

At the end of the starter phase, the different groups were carried through to the finishing stage. The same numbers of pens (12) were used for the finisher phase as in the starter phase with treatments replicated three times. The 20.5% crude protein broiler finisher diets were formulated to contain 0, 10, 20 and 30% RBDG respectively (Table 3.4). Feed and water were provided *ad libitum.* Mortality was recorded as it occurred. Weekly records of live weight gain, feed intake, feed to gain ratio and feed cost/kg gain were kept. The finisher phase lasted for 4weeks.

Table 3.3: Ingredient composition (%) of broiler starter diets containing graded levels of refermented brewers dried grain (0-4weeks)

Ingredients	Treatment			
	0%	**10%**	**20%**	**30%**
Maize	44.15	42.01	39.87	37.69
Maize offal	10.0	10.0	10.0	10.0
RBDG	0.0	10.0	20.0	30.0
G/Cake (44% CP)	35.6	27.65	19.7	11.8
Fish meal	4.0	4.0	4.0	4.0
Limestone	0.5	0.5	0.5	0.5
Bone meal	3.0	3.0	3.0	3.0
Palm oil	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3
Vit/M Premix[1]	0.3	0.3	0.3	0.3
L-Lysine	0.4	0.49	0.58	0.66
DL-Methionine	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.0**
Calculated Analysis				
Crude protein, (%)	23.01	23.01	23.01	23.01
ME, kcal/kg	2873	2853	2844	2829
Ether extract, (%)	5.87	6.41	6.96	7.51
Crude fibre, (%)	4.21	5.41	6.60	7.80
Calcium, (%)	1.25	1.26	1.28	1.29
Avail.P (%)	0.64	0.64	0.65	0.66
Lysine, (%)	1.25	1.25	1.25	1.25
Met. + cys. (%)	0.91	0.90	0.90	0.90
Feed cost (₦/kg)	45.68	44.03	42.38	40.65

[1]Vitamin/mineral premix (Animal Care-Optimix[R]) supplied per kg of feed: vit. **A** 5,000 i.u., **D_3** 3500 i.u., vit.k *2.5mg*, **B_1** 2mg, **B_2** 6mg, B_6 4mg, Niacin 40mg, B_{12} 0.02mg, Pantothenic acid 10mg, Folic acid 1mg. Biotin 0, 088mg, Choline chloride 0.5gm, Anti oxidant 0.125, Manganese 0.096gm. Iron 0.24gm, copper 3.60mg**,** iodine $1.4x10^{-3}$, Selenium 0.240mg and cobalt 0.240mg.

Table 3.4: Ingredient composition (%) of broiler finisher diets containing graded levels of refermented brewers dried grains (5-9 weeks)

Ingredients	Treatments			
	0%	**10%**	**20%**	**30%**
Maize	51.85	49.5	47.09	44.71
Maize offal	10.0	10.0	10.0	10.0
RBDG	0.0	10.0	20.0	30.0
G/Cake (44% CP)	28.3	20.57	12.9	5.2
Fish meal	3.8	3.8	3.8	3.8
Bone meal	3.0	3.0	3.0	3.0
Palm oil	1.7	1.7	1.7	1.7
Salt	0.3	0.3	0.3	0.3
Vit/M Premix[1]	0.3	0.3	0.3	0.3
L-Lysine	0.5	0.58	0.66	0.74
DL-Methionine	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis.				
Crude protein, (%)	20.50	20.49	20.50	20.50
ME: kcal/kg	2974	2955	2936	2917
Ether Extract, (%)	5.13	5.06	5.01	4.95
Crude fibre, (%)	4.32	5.10	5.88	6.66
Calcium, (%)	1.25	1.27	1.28	1.29
Available, P (%)	0.61	0.62	0.62	0.62
Lysine (%)	1.23	1.23	1.23	1.23
Met + cys (%)	0.85	0.86	0.88	0.90
Feed cost (₦/kg)	45.44	43.72	42.02	40.32

[1]Vitamin/ mineral premix (Animal Care.Optimix[R]) supplied per kg of feed: vit A 5,000 i.u., D_3 3500 i.u., vit.k *2.5mg*, B_1, 2mg, B_2 6mg, B_6 4mg, Niacin 4omg, B_{12} 0.02mg, Pantothenic acid 10mg. Folic acid 1mg. Biotin 0.088mg, Choline chloride 0.5gm, Anti oxidant 0.125, Manganese 0.096gm Iron 0.24gm., copper $6x10^4$mg, iodine $1.4x10^3$mg, Selenium 0.240mg, and cobalt 0.240mg.

3.7.3 Carcass characteristics

At the end of the broiler finisher phase (9-weeks), three birds from each pen whose weights were equal or close to the mean weight of the birds in the pen were selected and slaughtered for carcass analysis. The selected birds were slaughtered by severing the neck with a sharp knife and allowed to bleed completely. They were then de-feathered and eviscerated.

The breast, thigh, back, neck, wings, legs, head, liver, heart, gizzard, pancreas and the abdominal fat were weighed and expressed as percentages of the live weights. Intestinal lengths were measured in centimeters. The dressed weights were taken and the dressing percentages computed.

3.7.4 Data analysis

Data were subjected to analysis of variance for a completely randomized design. The statistical analyses were performed using the General Linear Model procedure of Statistical Analysis System (SAS) computer software package (1985*).* Differences between significant means were separated through the procedures of Duncan's Multiple Range Test (Steel and Torrie, 1980)

3.8.0 Refermentation of BDG

35kg of inoculum (rumen liquor) was first introduced into 1750kg of water. The same quantity of water (1750kg) was then used to wet 350kg of BDG.

This mixture represents water to substrate ratio of 5:1 (w/w) and inoculum to substrate ratio of 1:10 (w/w). The inoculated BDG was then carefully turned into 5-feet deep pit lined with polythene material. It was properly compacted by foot, and covered with polythene, soil and zinc sheets to maintain as much anaerobic condition as possible. The substrate (BDG) was allowed to ferment for two days. At the end of the fermentation, the pit was opened and observation was made on the colour, odor and consistency of products. The refermented BDG was then sun-dried for 7 days after which samples were taken for chemical analysis according to AOAC (1990) procedures. The results of the chemical analysis are as shown in table 4.7

3.8.1 The effect of lysine, methionine and enzyme supplementation on the performance and carcass characteristics of broiler chickens fed 30% RBDG diets.

Objectives:

Having observed from experiment 1 the depressed live weights and feed efficiency of broilers fed graded levels of RBDG (0, 10, 20 and 30%). The objectives of this study were:

1. To evaluate the performance of broiler chickens fed 30% RBDG diets with amino acids and enzyme supplementation.
2. To evaluate the effect of amino acid and enzyme supplementation on carcass characteristics of broiler chickens fed diets containing

30%RBDG.

3. To evaluate the nutrients metabolism by broiler chickens fed 30% RBDG diets.

3.8.2 The starter phase (0-4 weeks)

Three hundred and six day old-broiler chicks of mixed sexes and of Aboricae strain were used for this experiment. The chicks were weighed at day-old and randomly allocated to 18 pens in groups of 17 birds per pen. Six diets were randomly allocated to the pens. There were three replications for each of the treatments in a completely randomized design trial. The starter phase of the experiment lasted from zero to four weeks. The chicks were brooded conventionally in a deep-litter house. Kerosene lanterns and stoves were used as sources of heat. The electric power supply was ‘epileptic’ therefore lighting was most often natural. The starter diets (23.5% CP) were iso-nitrogenous but not isocaloric. The diets for the starter and finisher phases as shown in tables 3.5 and 3.6 were as follows;

Diet 1 was the control diet. It had no RBDG and no enzyme, but it had lysine and methionine added to meet the minimum NRC requirement. Diet 2 contained 30% RBDG but without enzyme supplementation. The diet had Lysine and methionine added to meet the minimum NRC requirement. Diet 3 contained 30% RBDG plus lysine slightly above NRC requirement. Diet 4

contained 30% RBDG plus methionine slightly above NRC requirement. Diet 5 contained 30% RBDG, meeting NRC requirement for Lysine and methionine plus 0.02% enzyme (Allzyme SSF) supplementation. Diet 6 contained 30% RBDG plus lysine and methionine slightly above the NRC recommended values plus 0.02% enzyme (Allzyme SSF) supplementation.

The enzyme used in this experiment was Allzyme SSF (Alltech Inc Nicholasville KY). Allzyme SSF is an enzyme complex, which has the following enzyme activities; Amylase, Cellulase, Phytase, Xylanase, Pectinase, Protease, and Beta-Glucanase.

The enzyme was supplemented at the level of 200g/ton of feed as recommended by the manufacturers. The treatments were arranged in a completely randomized design. Birds were provided with feed and water *ad libitum*. Mortality was recorded as it occurred. The weights of birds and of leftover feeds were taken weekly. Live weight, average daily gain, feed intake, feed to gain ratio and feed cost per kilogram gain (₦) were determined for the period.

3.8.3 Finisher Phase (5-9weeks)

At the end of the starter phase, the birds were drawn together and fed on a common diet for one week. Two hundred and thirty four (234) birds were

randomly re-assigned to the experimental pens, balancing for mean weights in each of the 18 experimental pens. There were also six treatments for the finisher phase. The treatments were similar to those employed in the starter phase. Each of the treatment was replicated three times with thirteen (13) birds per pen. The finisher test rations were formulated to be iso-nitrogenous at 21.5% CP. The composition of the finisher diets is as shown in table 3.7. Feed and water were supplied *ad libitum.* Mortality was recorded as it occurred. Weekly records of live weight, weight gain, average daily gain, feed intake, feed to gain ratio and feed cost per kg gain was kept. The finisher phase lasted from 5 to 9 weeks.

Table 3.5: Ingredient composition (%) of RBDG diets supplemented with amino acids and enzyme (starter phase)

Diets **Ingredients**	**1** **0%** **RBDG**	**2** **30%** **RBDG**	**3** **30% RBDG** **+ lys**	**4** **30% RBDG** **+ Met**	**5** **30%RBDG** **+ Enz**	**6** **30%** **RBDG** **+ LME**
Maize	47.48	35.24	35.05	35.02	35.23	34.80
RBDG	0.0	30.0	30.0	30.0	30.0	30.0
PKC	5.0	5.0	5.0	5.0	5.0	5.0
GNC 44%	30.3	9.73	9.77	9.77	9.72	9.82
SBC	8.0	8.0	8.0	8.0	8.0	8.0
Fish meal	3.5	3.5	3.5	3.5	3.5	3.5
Palm oil	1.5	4.5	4.5	4.5	4.5	4.5
Bone Meal	1.05	1.09	1.09	1.09	1.09	1.09
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Premix[1]	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0.19	0.38	**0.53**	0.38	0.38	**0.53**
Methionine	0.23	0.17	0.17	**0.35**	0.17	**0.35**
Limestone	1.95	1.79	1.79	1.79	1.79	1.79
Enzyme	0.00	0.00	0.00	0.00	**0.02**	**0.02**
Total	100	100	100	100	100	100
Calculated Analysis						
CP, %	23.52	23.52	23.52	23.51	23.51	23.52
ME, kcal/kg	2884	2820	2820	2814	2819	2807
EE, %	5.67	4.91	4.91	4.91	4.91	4.90
CF,%	4.26	7.06	7.05	7.05	7.06	7.04
Ca,%	1.27	1.28	1.28	1.28	1.28	1.28
Avail,P	0.62	0.62	0.62	0.62	0.62	0.62
Lysine, %	1.21	1.20	**1.35**	1.20	1.20	**1.35**
Met+Cys,%	0.85	0.85	0.85	**1.03**	0.85	**1.03**
Feed cost (₦/kg)	33.56	34.92	35.76	36.02	35.04	1.03

Premix[1] vitamin premix (Animal Care Optimix[R]) supplied per kg of feed: vit A 5,000 i.u, D_3 3500 i.u., vit.k 2.5mg, B_1 2mg, B_2 6mg, B_6 4gm, Niacin 40mgm, B_{12} 0.02mg, Pantothenic acid 10mg, Folic acid 1mg, Biotin 0, 088mg, Choline chloride 0.5gm, Anti oxidant 0.125mg, Manganese 0.096gm. Iron 0.24gm., copper $6x10^3$mg, iodine $1.4x10^3$mg, selenium 0.240mg, and cobalt 0.240mg

Table 3.8: Ingredients composition (%) of RBDG diets supplemented with amino acids and enzyme (Finisher phase)

Diets Ingredients	1 0% **RBDG**	2 30% **RBDG**	3 30% RBDG **+ lys**	4 30% RBDG **+ Met**	5 30%RBDG **+ Enz**	6 30% **RBDG + LME**
Maize	53.16	39.35	39.10	39.10	39.33	38.83
RBDG	0.00	30.00	30.00	30.00	30.00	30.00
PKC	5.00	5.00	5.00	5.00	5.00	5.00
GNC 44%	25.92	5.63	5.68	5.68	5.63	5.73
SBC	7	7	7	7	7	7
Fish meal	3.5	3.5	3.5	3.5	3.5	3.5
Palm oil	1.5	5.5	5.5	5.5	5.5	5.5
Bone meal	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Premix[1]	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0.1	0.26	**0.46**	0.26	0.26	**0.46**
Methionine	0.22	0.16	0.16	**0.36**	0.16	**0.36**
Limestone	0.5	0.5	0.5	0.5	0.5	0.5
Enzyme	0	0	0	0	**0.02**	**0.02**
Total	100	100	100	100	100	100
Calculated Analysis						
CP, %	21.54	21.54	21.54	21.54	21.54	21.54
ME, kcal/kg	2941.53	2918.74	2911.39	2911.39	2918.05	2903.35
EE, %	5.43	4.65	4.64	4.64	4.65	4.64
CF,%	4.27	7.01	7.00	7.00	7.01	7.00
Ca,%	1.30	1.33	1.33	1.33	1.33	1.33
Avail,P	0.82	0.82	0.82	0.82	0.82	0.82
Lysine, %	1.03	1.00	**1.20**	1.00	1.00	**1.20**
Met. + Cys(%)	0.80	0.80	0.80	**1.00**	0.80	**1.00**
Feedcost (₦/kg)	31.64	34.64	35.77	37.87	34.76	36.99

Premix[1] vitamin premix (Animal Care Optimix^{R}) supplied per kg of feed: vit A. 5,000 i.u., D_3 3500 i.u.,vit 2.5mg, B_1 2mg B_2 6mg B_6 4mg, Niacin 40gm, B12 0.02mg,Pantothenic acid 10mg Folic acid 1mg, Biotin 0.88mg, chloride O.5gm, Anti oxidant 0.125mg, Manganese 0.096gm, Iron O.24gm, copper $6x10^{-3}$mg, Iodine $1.4x10^{-3}$mg, Selenium 0.240mg,

3.8.4 Carcass characteristics of finished broilers

At the end of the nine-week feeding trial, two birds from each pen with approximate body weight equal to the mean weight of the birds in the pen were selected and weighed. The selected birds were slaughtered for carcass analysis by severing the jugular vein and structures of the neck with a sharp knife. The birds were allowed to bleed completely, defeathered and eviscerated. The weight of the breast, thigh, back, neck, wing, legs, head, liver, heart, gizzard, pancreas and abdominal fat were recorded. These were expressed as percentages of live weight. The intestinal length was measured in centimeters. The dressed weights were taken and the dressing percentage computed.

3.8.5 Nutrient metabolism of RDBG diets supplemented with synthetic amino acids and enzyme

Procedure

At the end of the feeding trial, eighteen broiler chickens of average initial weight of' 2.55kg was retained from each of the 18 pens housing broiler chickens. There were replications of the 6 treatments in a completely randomized design and birds were fed their respective finisher diets. Birds were assigned to individual wire cages ($1.3m^2$) in each pen. Trays were placed under each cage for faecal collection. Water and feed were made available

ad libitum throughout the 7- day trial period time. Care was taken so that the total number of birds (18) had equal number of males and females in each treatment.

The first ration fed to the birds at the commencement of the 7-day experiment and the last ration fed at the end of the experiment contained an indigestible colored substance (soot) used as marker. The beginning and the end of faecal collection were delayed until the marker (black color of soot) appeared in and disappeared from the excreta. Faeces were collected daily, air-dried, weighed and then pooled and stored in a freezer for the 7-day period. The faeces were thawed, thoroughly mixed and samples obtained for laboratory analysis. Samples of the six diets and faeces were then taken for proximate analysis according to the procedures of' the Association of Official Analytical Chemist (A.O.A.C, 1990).

The quantities of nutrients in the feed and faecal samples were determined from the percentage nutrients obtained from chemical analysis and the record of feed intake and faecal output. Nutrient metabolism was determined as follows;

$$\text{Percentage nutrient metabolism} = \frac{D_e \text{ x } \%\, N_d - D_f \text{ x } \%N_f \text{ x } 100}{D_e \, x \, N_d}$$

Where D_e = dry weight of diet eaten

$\%N_d$ = Percentage nutrient in the diet

D_f = dry weight of faeces voided

$\%N_f$ = Percentage nutrient in the faeces

3.8.6 Data analysis

Data were subjected to analysis of variance for a completely randomized design. The statistical analysis was performed using the General Linear Model procedure of Statistical Analysis System (SAS) computer software package (1985). Differences between significant means were separated through the procedures of Duncan's Multiple Range Test (Steel and Torrie, 1980)

CHAPTER FOUR

RESULTS

4.1.1 Laboratories Studies

4.1.2 The effect of re-fermentation and nutrient composition of BDG

4.1.3 Nutrient composition of RBDG

The proximate composition of BDG analyzed before and after fermentation are as shown in table 4.2.

Table 4.1. Proximate composition of BDG and [1]RBDG analyzed

Analysis *	Ingredients	
	BDG	[1]RBDG
[2]Dry Matter (%)	95.10	95.41
Ash (%)	4.98	4.98
Ether Extract (%)	9.44	9.44
Nitrogen Free Extract	23.28	30.95
Crude Fibre (%)	37.90	26.63
Crude Protein (%)	24.44	28.00

*Values are means of two determinations
[1]Uninoculated fermented BDG sample.
[2]Analysis % of DM

The wetted uninoculated BDG sample fermented for 8-days also revealed an increased crude protein content from 24.44 to 28.00% (Table 4.1). Compared to the crude protein content of all the re-fermented inoculated BDG samples (Table 4.2), the wetted uninoculated BDG sample was observed to contain the least crude protein content (28.0 %).

The crude protein content of BDG was observed to increase from 24.44% before fermentation to between 29.28-36.28 % after fermentation (Table 4.2). The RBDG sample with the highest crude protein (36.28%) was observed among samples on 2-day fermentation with substrate to inoculum concentration ratio of 10:2

The crude fibre content was observed to decrease from 37.90% before fermentation to 14.16% after fermentation. The least crude fibre content was observed among samples on 2-day fermentation and with the sample of substrate to inoculum concentration ratio of 10:1. The crude fibre content of the wet uninoculated fermented BDG sample was observed to be lower than the normal dried BDG sample but higher than all the inoculated samples.

4.1.4 Effect of different inoculum concentration on nutrient composition of refermented BDG

Table 4.3 shows the effect of BDG re-fermentation with different levels of inoculum concentrations on the nutrient composition of BDG. In terms of dry matter composition, all BDG samples with substrate to inoculum concentration of 10:2, 10:3 and 10:4 on fermentation did not show any significant difference among the group. BDG samp1e on 10:5 and 10:1 substrate to inoculum concentration were similar but significantly higher ($P<0.05$) than samples on, 10:2, 10:3 and 10:4 substrate to inoculums

concentration. The control sample was significantly higher in percentage DM than other samples on different inoculum concentration.

Table 4.3: Effect of different BDG re-fermentation inoculum concentrations on BDG nutrient composition.

	Substrate to inoculum concentration						
Items	control	10:1	10:2	10:3	10:4	10:5	SEM
DM, %	95.1^{a}	93.59^{b}	93.52^{c}	93.13^{c}	93.63^{c}	94.41^{b}	0.25
CP,%	24.44^{c}	34.39^{a}	34.97^{a}	34.11^{a}	31.86^{b}	33.91^{a}	0.42
EE,%	9.04^{c}	14.71^{a}	11.84^{b}	11.60^{b}	10.09^{b}	10.10^{b}	0.62
CF, %	39.0^{a}	15.59^{d}	16.69^{c}	17.85^{b}	16.98^{bc}	16.30^{cd}	0.34
ASH,%	4.07^{b}	4.03^{b}	0.48^{b}	4.41^{b}	4.52^{ab}	4.99^{a}	0.16

^{a,b,c,d} Means of the same row with different superscripts are significantly different ($P<0.05$)

The crude protein content of all RBDG samples were significantly higher ($P<0.05$) than the unrefermented BDG sample. The crude protein content of samples on 10:1, 10:2, 10:3 and 10:5 were similar but significantly higher ($P<0.05$) than that of the BDG sample on 10:4. The percentage ether extract of all RBDG samples were significantly higher ($P<0.05$) than the control. The percentage ether extract of samples on 10:2, 10:3, 10:4 and 10:5 substrate to inoculum concentration were similar but significantly lower ($P<0.05$) than those on 10:1. The percentage crude fibre of the control sample was significantly higher ($P<0.05$) than that of all RBDG samples. The crude fibre composition of samples on 10:2, 10:4 and 10:5 were similar ($P>0.05$).

Samples on 10:3 substrate to inoculum concentration contained higher percentage crude fibre ($P<0.05$) than samples on 10:1, 10:2 and 10:5. Samples on 10:1 had significantly ($P<0.05$) lowest percentage of crude fibre which was similar to samples on 10:5 substrate to inoculum concentration.

The percentage ash for the control sample and that of samples on 10:1, 10:2, 10:3 and 10:4 substrate to inoculum concentrations were similar. The ash content of BDG sample on 10:5 was significantly higher ($P<0.05$) than those on 10:1, 10:2 and 10:3. Samples with 10:4 and 10:5 concentrations had similar percentage ash.

4.1.5 Effect of different periods of fermentation of BDG on nutrient composition

Table 4.4 shows the effect of different periods of re-fermentation on the nutrient composition of BDG. The dry matter composition was significantly higher ($P<0.05$) for 2-day fermentation compared to those on 4, 6 and 8-day fermentation but significantly lower ($P<0.05$) to the control. The dry matter for samples on 6 and 8-day fermentation were similar but significantly lower ($P<0.05$) than that of 4-day fermentation.

Table 4.4: Effect of period of re-fermentation on nutrient composition of BDG.

	Fermentation period (days)					
Items	0	2	4	6	8	SEM
DM (%)	95.1	94.81	93.99	92.98	92.83	0.22
ASH (%)	4.07^{b}	4.76^{a}	4.49^{a}	4.32^{a}	4.38^{a}	0.15
EE (%)	9.04^{c}	8.66^{c}	14.81^{a}	11.75^{b}	11.45^{b}	0.55
CF (%)	37.90^{a}	16.28^{c}	16.47^{c}	16.23^{c}	17.75^{b}	0.30
N (%)	3.91^{c}	5.42^{ab}	5.32^{b}	5.53^{a}	5.38^{ab}	0.06
CP (%)	24.44^{c}	33.88^{ab}	33.28^{b}	34.59^{a}	33.65^{ab}	0.38

^{a,b,c}Means within row with different superscripts are significantly different (P<O.05)

There was no significant difference (P>0.05) in the ash composition across samples for the different period of fermentation. The percentage ash composition of the control sample was significantly lower than those of samples on different period of fermentation. The ether extract for 4-days fermentation was significantly higher (P<0.05) compared to those of 2, 6 and 8-day fermentation periods and the control. The ether extract content of 2-day fermentation was also significantly lower (P<0.05) than those on other fermentation periods. Samples on 2, 4 and 6-day fermentation had similar crude fibre content and were significantly lower (P<0.05) than the crude fibre content of the 8-day fermentation sample. The percentage crude protein content of the control sample was significantly (P<0.05) less than those of samples on different periods of fermentation. The crude protein content of

samples on 6-day fermentation was similar to those on 2 and 8-day but significantly higher than those on 4-day fermentation. The crude protein content of the control was significantly the lowest.

Table 4.5 shows the effect of BDG fermented for different periods with different inoculum concentration on crude protein and crude fibre compositions. The 2-day fermented samples on treatments 10:1 and 10:2 substrate to inoculum concentration did not differ significantly ($P>0.05$) in crude protein composition. Sample on 10:2 had higher ($P<0.05$) % CP compared to those that were on 10:3, 10:4 and 10:5. Sample on treatment 10:4 had the least % CP ($P<0.05$). BDG samples on 10:1, 10:2, 10:3, 10:4 and 10:5 inoculum concentration and of 4, 6 and 8-days fermentation did not differ ($P>0.05$)in their crude protein content.

On the basis of crude fibre composition, all RBDG samples on 2-day fermentation with different inoculum concentrations did not differ significantly ($P>0.05$). Similarly RBDG samples on 4, 6 and 8-day fermentation with different inoculun concentration did not differ significantly ($P>0.05$) in crude fibre content.

Table 4.5: Effect of fermentation on BDG crude protein and crude fibre composition

	Concentration					
Number of days	10:1	10:2	10:3	10:4	10:5	SEM
	Crude Protein ,%					
2	34.47^{ab}	36.38^{a}	33.26^{b}	29.75^{c}	33.89^{b}	0.29
4	34.69	33.44	34.63	33.94	33.10	0.98
6	33.89	35.00	34.94	35.00	34.01	0.40
8	34.76	35.16	33.40	29.38	31.00	1.60
	Crude fibre ,%					
2	14.61	17.21	16.43	17.29	15.89	0.69
4	16.09	15.56	18.36	16.08	16.22	0.64
6	16.26	16.65	18.23	15.71	15.09	0.98
8	16.17	17.34	18.36	18.86	18.02	0.64

^{a,b,c} Means within row with different superscript are significantly different ($P<0.05$)

4.1.6 Bacteria count on plates

The result of the laboratory bacteria count is as shown in table 4.6. There was no definite trend observed in the bacteria population between samples in the different fermentation periods (2, 4, 6 and 8days).

4.1.7 Microscopy

The result of the microscopic examination revealed the presence of gram-positive short chain cocci and gram-negative purple rod shaped bacteria.

4.2.0 On-farm preparation of re-fermented brewers' dried grains

The results of the chemical analysis carried out after bulk BDG re-fermentation for use in feeding trials are as shown in table 4.7.

Table 4.7.proximate composition of BDG and RBDG analysed

Analysis*	BDG	[1]RBDG	[2]RBDG
Dry matter (%)	95.10	96.07	95.80
Ash (%)	4.98	4.52	4.53
Ether extract (%)	9.44	6.87	5.73
Nitrogen free extract	23.24	37.52	42.29
Crude fibre (%)	37.90	14.68	13.59
Crude protein (%)	24.44	36.41	33.86

*Values are means of two determinations
[1]RBDG sample fermented for use in experiment i
[2]RBDG sample fermented for use in experiment ii

4.3.0 Effect of feeding graded levels of RBDG on the performance and carcass characteristic of broiler chickens

4.3.1 Starter Period (0-4 weeks)

The performance of broiler chicks on the experimental diets during the starter phase is as shown in Table 4.8. Daily feed intake of birds on 30% RBDG was significantly higher ($P<0.05$) than for other treatments. The daily feed intake of birds on 10% and 20% were similar ($P>0.05$) but significantly higher ($P<0.05$) than those on the control diet. Feed intake increased with increasing RBDG level. Average daily weight gain for the birds on control was similar to those on 10% and 30% RBDG diets, but significantly higher ($P<0.05$) than

those on 20% RBDG diets. The daily weight gain of birds on 10, 20 and 30% RBDG were similar (P<0.05).

The feed to gain ratio of birds on 20% and 30% RBDG diets were similar (P>0.05) but significantly higher (P<0.05) than those on 10% RBDG and the control. Feed to gain ratio of birds on 10% RBDG diet was significantly higher than those on the control diet. Feed to gain ratio was observed to increase with increasing RBDG level up to 20%.

The cost of feed/kg gain (₦) during the starter phase for birds on control and 10% RBDG diets were significantly lower (P<0.05) compared to those of birds on 20 and 30% RBDG diets which were similar. The feed cost/kg gain for birds on 10% RDBG diet was significantly higher (P<.0.05) than that for birds on the control diet.

During the starter phase mortality was significantly higher (P<0.05) for birds on 30% RBDG diet than for other diets with no significant differences (P>0.05) among other diets.

4.3.2 Feeding trial (0-9 weeks,)

Observations on the performance of the birds from the commencement to the end of the trial (0-9weeks, Table 4.9) revealed significant differences (P<0.05) in performance parameters across the treatments. Birds in the 30% RBDG diet consumed significantly (P<0.05) more feed compared to those on other diets.

Daily feed intake increased linearly with increased level of RBDG in the diet. Weight gain was significantly higher ($P<0.05$) for birds on the control diet than those on other diets. Weight gains of birds on 10% RBDG diet was also significantly higher ($P<0.05$) compared to those on 20 and 30% RBDG diets which were similar. The gains of birds on the 20 and 30 % RBDG diets were similar. Weight gain was generally observed to reduce with increasing level of RBDG in the diet.

The feed to gain ratio of birds on 30% RBDG diet was significantly ($P<0.05$) the highest among all the diets. Thus the 30% RBDG diet was poorly utilized for gain compared to the control and other RBDG diets. Birds on the 20% RBDG diet had significantly higher ($P<0.05$) feed to gain ratio compared to birds on 10% RBDG diet. Feed efficiency revealed a downward trend as dietary level of RBDG increased.

4.3.3 Carcass characteristics

Table 4.11 shows the result of carcass analysis and the organ weights of the experimental birds. There were no significant differences ($P>0.05$) in dressing percentage, thigh and leg percentages among birds on 0, 10 and 20% RBDG. The percentage breast and back were not significantly affected ($P>0.05$) by the level of RBDG fed in the diet. The dressing percentage, thigh and leg percentages of birds on 30% RBDG diets were similar to those of birds on the

control and 10% RBDG diets, but were significantly lower ($P<0.05$) compared to those on 20% RBDG diet. The percentage, head and neck of the birds across the treatment groups did not differ significantly ($P>0.05$). Similarly the gizzard, heart, pancreas and abdominal fat weight were not affected by dietary treatments. The percentages for wings and liver of birds on the 30% RBDG diets were significantly higher ($P < 0.05$) than those of birds on control, 10 and 20% RBDG diets. The percentage liver and wings of birds on control, 10 and 20% were similar ($P>0.05$).

4.4.0 The effect of lysine, methionine and enzyme supplementation of 30% RBDG diet on performance and carcass characteristics of broiler chickens.

4.4.1 Broiler starter phase (0-4 weeks)

The results of the performance of broiler chicks fed the control and unsupplemented or the supplemented 30% RBDG diets during the starter phase are as shown in Table 4.11. The daily gain of birds on amino acids and enzyme- supplemented diet (+LME) were significantly higher ($P<0.05$) than those of birds on unsupplemented 30% RBDG diet. Daily feed intake increased with amino acid and enzyme supplementations. Feed intake of birds on diets; 3 (+ Lys), 4 (+ met), 5 (+Enz) and 6 (+LME) were similar but significantly higher ($P<0.05$) than those on the control diet.

Total and daily feed intake of birds on the supplemented diets were similar and were significantly higher ($P<0.05$) than those on the control diet. There was no significant difference ($P>0.05$) in feed cost per kg gain for birds on 30% RBDG diet and those on amino acid and enzyme supplemented diets (+ lys, + met and +Enz). Feed cost/kg gain for birds on control and 30% RBDG + LME diets were similar but significantly cheaper ($P<0.05$) than those of 30% RBDG, 30% RBDG + lys, 30% RBDG +Met and 30% RBDG + Enz diets.

The rate of mortality of birds on the different diets was also differed. The

mortality rate for birds on the control diet and those on unsupplemented diet, + lys and + Enz diets were similar (P>0.05). The mortality rates for birds on 30% RBDG + Met and 30% RBDG + LME diets were similar but significantly lower (P<0.05) than those of birds on control and 30% RBDG, 30% RBDG + lys, and 30% RBDG + Enz diets.

4.4.2 Broiler finisher phase (5-9 week)

The results of the performance of broilers fed 30% RBDG with and without amino acid and enzyme supplemented diets during the finisher phase are presented in Table 4.12. Dietary treatments during the finisher phase had significant effect (P<0.05) on final weights, average final weight, total weight gain, and daily weight gain, feed intake, feed to gain ratio, feed cost per kg gain and on mortality. The final and daily weight gains of birds on control and those on 30% RBDG + lys, 30% RBDG + met and 30% RBDG + Enz diets were observed to be similar (P>0.05). The daily weight gain of birds on 30% RBDG + LME was similar to those on control and 30 % RBDG + lys diets but significantly higher (P<0.05) when compared to those of birds on unsupplemented, 30% RBDG + Met and 30% RBDG + Enz diets.

The feed intake of birds on RBDG diet supplemented with amino acids and enzyme (+LME) were significantly higher ($P<0.05$) than those of birds on un-

supplemented RBDG diet and other diets containing only the individual amino acid or enzyme. The daily feed intake was observed to be similar for birds on the control diet and those on unsupplemented, + lys, + Met and +Enz diets. The total and daily feed intake of birds on 30% RBDG + Enz diet was observed to be significantly higher ($P<0.05$) than those on 30% RBDG + lys diet.

Feed to gain ratio of birds on unsupplemented diet was significantly higher than those on the control and also those on 30% RBDG + lys, + Met, + Enz and + LME diets. The supplementation of 30% RBDG with lysine alone resulted in improved feed to gain ratio similar to that of the control birds. The supplementation with methionine, plus enzyme and plus lysine only resulted in feed to gain ratio similar to that of control and Lysine alone. The cost of feed per kg gain (₦) for birds on the control diet was significantly the lowest ($P<0.05$) among all treatments. The feed cost per kg gain of birds on 30% RBDG +lysine diet was significantly lower ($P<0.05$) than those on other diets. The feed cost per kg gain for 30% RBDG + Met, +Enz and + LME diets were similar. The feed cost per kg gain of birds on 30% BDG + lysine diet was significantly lower ($P<0.05$) compared to those on unsupplemented + Met, + Enz and + LME diets but significantly higher ($P<0.05$) than those on control diet.

The mortality rate was observed to be similar (P>0.05) among birds on control and unsupplemented diet. The rate of mortality was highest P<0.05) for birds on + Met diet, followed by those on + lys and + Enz diets. Mortality on + lys and +Enz diets were also higher than those on the control and unsupplemented diet. There was no mortality recorded among birds on 30% RBDG + LME diet during the finisher period.

4.4.4 Carcass characteristics

Table 4.16 shows the results of carcass analysis of the experimented bird..

Dressing percentages for birds on all diets were similar (P>0.05). The percentage thigh for birds on unsupplemented, 30% RBDG + Met, 30% RBDG + LME and control diets were similar and significantly higher (P<0.05) than those on 30% RBDG + lys diet . Birds on 30% RBDG + lys and + Enz diets had similar percentage thigh (P>0.05). Percentage back for birds on 30% RBDG + Enz and + LME diets were similar (P>0.05), but higher than percent back for control, unsupplemented, + lys, and 30% RBDG + Met diets. Significant differences (P>0.05) were not observed for percentage liver for birds on 30% RBDG + lys, + Met, + Enz and the control diets. Birds on unsupplemented diet had significantly higher (P<0.05) percentage liver than

those on 30% RBDG + LME diet but lower significantly than those on +Lys, + Met, + Enz and control diets. The percentage gizzard was observed to be

similar between birds on 30% RBDG + lys, + Enz and + LME diets Similarly, birds on + LME and the control diet had similar percentage gizzard. There was no significant difference between birds on unsupplemented, and + Met diets. These also had the highest ($P<0.05$) gizzard weights. The control diet had the lowest ($P<0.05$) gizzard weights

Percentage heart for birds on the control, + lys and + Enz diets were similar ($P>0.05$). Those of birds on + Met and + LME diets were also similar ($P>0.05$). Birds on + Met and + LME diets had significantly the highest percentage heart while those on the control diet had the lowest ($P<0.05$). There was no significant difference ($P>0.05$) for the percentage abdominal fat between birds on + lys and + Met diets and also between birds on unsupplemented and + Enz diet diets. Birds on + LME diet had significantly the highest percent abdominal fat ($P<0.05$) while those on unsupplemented and + Enz diets had the lowest percent abdominal fat ($P<0.050$). Birds on + lys and + Met diets had lower abdominal fat compared to those on the control diet The intestinal length of birds on unsupplemented and + Met diets were similar. Birds on + lys and + Enz diets had similar intestinal length. Birds on + Lys diet had the lowest ($P<0.05$) intestinal length. Birds on + lys and control diets had significantly longer intestinal length compared to those on unsupplemented and + Met diets.

4.5.0 Metabolism of nutrients in RBDG diets supplemented with synthetic amino acids and enzyme.

Tables 4.15 and 4.16 show the results of the chemical composition of the experimental diets. Table 14.17 shows the results of dry matter and ash, ether extract and nitrogen and energy metabolism by birds fed the experimental diets for 7 days.

4.5.1 Nutrient Metabolism

There were no significant differences in the amount of dry matter (DM) consumed by birds on the lysine and methionine supplemented diets and un-supplemented diet (30% RBDG) compared to those on the control diet. The daily DM consumed by birds on enzyme supplemented diet (+Enz) and combined amino acids and enzyme supplemented diet (+LME) were similar and significantly higher ($P< 0.05$) than those on the control diet (0% RBDG). The DM intake of birds on the control diet was significantly lower ($P<0.05$) compared to that of birds on other diets. The daily DM digested by birds on lysine and methionine supplemented diets and the unsupplemented diet were similar to the control diet ($P>0.05$) but significantly lower ($P<0.05$) than those digested by birds on + Enz amid +LME diets. There was no significant

difference in DM metabolism between the RBDG + Enz and RBDG + LME. The apparent metabolizable DM of RBDG +Enz and RBDG +LME diets were

Table 4.15: Proximate composition of test diets analysed

Items	Treatments					
	0% RBDG	30% RBDG	30%+ Lys	30%+ Met	30%+ ENZ	30%+ LME
Dry matter	96.23	96.11	96.76	96.51	96.45	96.01
CP,%	22.55	23.12	23.46	21.88	22.13	22.09
CF, (%)	5.39	9.08	8.64	8.85	7.32	8.06
Ash, (%)	10.51	7.65	8.45	8.53	9.15	7.38
EE, (%)	10.35	8.85	8.99	8.75	7.55	8.90
N, (%)	3.61	3.71	3.58	3.50	3.54	3.55
G.E kcal/g	3.95	3.25	3.45	3.59	3.51	3.55

Results are means of two determinations

Table 4.16: Proximate composition of faecal droppings analysed

Items	Treatments					
	0% RBDG	30% RBDG	30%+ Lys	30%+ Met	30%+ ENZ	30%+ LME
Dry Matter, (%)	96.47	96.05	96.46	95.54	96.98	96.57
CP,%	10.62	16.03	12.04	13.03	10.75	9.38
CF, (%)	3.07	5.81	4.07	5.95	3.56	3.32
Ash, (%)	5.22	5.87	4.86	6.29	5.12	3.85
EE, (%)	4.85	5.66	4.26	5.38	3.63	3.56
N, (%)	1.62	2.51	1.94	2.10	1.71	1.55
G.E kcal/g	1.13	2.14	1.98	2.12	1.86	1.95

Results are means of two determinations

similar to each other ($P<0.05$).The apparent metabolizable DM of the 30% RBDG diet was similar to that of methionine supplemented diet but significantly lower than the control and other diets.

The daily nitrogen consumed by birds on unsupplemented 30% RBDG, + Lys, + Met and + Enz diets were similar to those on control. The nitrogen intake by birds on + LME diet was significantly higher than those on other diets but similar to the nitrogen intake for birds on + Enz diet. Similarly, the nitrogen digested by birds on + Lys, + Met and the unsupplemented 30% RBDG diets were similar to those on the control diet. There were no significant differences ($P>0.05$) in nitrogen digested by birds on + Lys, and +Met diets. The nitrogen digested by birds on + LME diet was significantly higher ($P<0.05$) than those on the other diets. The apparent metabolizable crude protein were similar for birds on the control + Lys, + Met, +Enz and +LME diets. The apparent metabolizable protein of 30% RBDG and +Met diet were similar ($P>0.05$). The apparent metabolizable protein was highest for the +LME diet followed by that for the control diet. The lowest metabolizable crude protein was observed in unsupplemented 30% RBDG diet.

Daily gross energy (GE) intake was similar for birds on the control and those on other diets with the exception of those on +LME diet. The excreted energy was similar for birds on + Lys and those on control diet. The energy voided by

birds on + Met and + Enz were similar but lower than the energy voided by birds on the unsupplemented 30% RBDG diet and also those on + LME diet. There were no significant differences ($P>0.05$) in daily energy and apparent energy metabolized by birds across dietary treatments.

On the basis of daily ash intake, there were no significant differences ($P>0.05$) between the ash consumption of birds on the control and those 30% RBDG and those on lysine and methionine supplemented diets. The ash intake by birds on enzyme supplemented diet and those on +LME were similar but significantly higher than those on control and other diets. The significantly highest ($P<0.05$) daily ash was voided by birds on the unsupplemented diet and lowest significantly ($P<0.05$) by birds on the 30% RBDG +LME.

The daily ash voided by birds on RBDG +Met and RBDG +Enz were similar but significantly higher than that voided by birds on the control. The daily ash digestion by birds was similar for both + Enz and + LME diets which were significantly higher ($P<0.05$) than the ash digestion for birds on the other diets. The apparent metabolizable ash of lysine, enzyme supplemented and the + LME diets were similar to that of the control diet. The metabolized ash of the unsupplemented 30% RBDG diet and the methionine supplemented diets were similar but were significantly lower ($P<0.05$) than the control and other diets.

The ether extract digested by birds on the control diet was similar to those of birds on other diets but significantly lower ($P<0.05$) than EE digested by birds on +LME supplemented diet. The apparent metabolizable EE did not differ significantly ($P>0.05$) for +Lys, +Enz and + LME diets as compared to the control diet. The EE metabolism of +Met and + Enz diets were similar to those obtained for the 30% unsupplemented RBDG diet. The highest EE digestibility was observed in birds on +LME diet followed by that of birds on the control diet. The lowest EE metabolism was observed in birds on the 30% RBDG diet.

The CF metabolized by birds on + Enz diet was similar to those on +Lys and +LME diets. The CF metabolized by birds on + Lys and +Met diets were similar to those on the unsupplemented 30% RBDG diet. There were no significant differences ($P>0.05$) in apparent metabolizable CF among birds on + Lys. +Enz, +LME and the control diets. The CF metabolism of the unsupplemented 30% RBDG diet was similar to that of + met diet. Birds on +LME and + Enz were observed to have the highest metabolizable CF, followed by birds on the control diet. The least CF metabolized diet was observed in birds on the unsuplemented 30% RBDG.

CHAPTER FIVE

5.0 DISCUSSION

5.1.0 Effect of feeding graded levels of RBDG on performance and carcass characteristics of broiler chicks

Fermentation caused a slight decrease in the dry matter percentage of RBDG. This did agree with the findings of Odufa (1987) who reported a reduction in the dry matter percentage during fermentation. Tyokpat (1989) did not observe any effect on the percentage dry matter after fermentation. These differences could probably be as a result of the differences in the fermentation periods used.

The increased percent crude protein and the decrease in percent crude fibre of BDG after fermentation respectively in this study were in agreement with the findings of Adeyemi and Adeleke (2000) that showed an increase in %CP of thevetia cake after fermentation. Similarly, Ofuya and Obilor (1993) reported an increase in %CP value of Cassava peel after 20 days fermentation.

The use of rumen liquor as inoculums for the fermentation of BDG resulted in significant increase in the crude protein content from 24.44 to 36.41%. This rate of increase was higher than the 7.74% increase observed by Oyegbile (1988). This observation may be attributed to the differences in fermentation conditions. In this study fermentation was effected outdoors where macro-

climatic effect may have differed from the indoor climate under which spent sorghum mash (SSM) was fermented.

The general increase in feed intake of birds across the treatment groups with increase in the dietary level of RBDG could be attributed to the increased CF content of the diet. As the inclusion level of RBGD increased, there was a reduction in the metabolizable energy value of the diets. Thus birds had to increase their feed intake to satisfy their energy requirements. This is in agreement with Dairo (1999) who observed significantly increased average daily feed intake and feed conversion ratio as the level of sorghum distillers waste inclusion increased in the diets.

Increase in daily feed intake observed as dietary level of RBDG increased in this study did not agree with Yaakugh *et al*., (1994) who observed a linear decrease in feed intake as the level of maize replaced by BDG increased.

There was a reduction in daily weight gain of birds with increasing RBDG inclusion. This revealed the superiority in the quality of the control diet which contained higher levels of groundnut cake than diets containing RBDG. The reduction in daily weight gains of birds as the RBDG inclusion levels increased was in agreement with the findings of Yaakugh *et al.*, (1994) who observed a linear decrease in average daily gain as the level of maize replaced by BDG increased in the diet.

The feed to gain ratio increased as the level of RBDG increased in the diet. The probable reasons here could be as a result of nutrient inadequacy and decreased energy content of the diet with increased RDBG in the diet. The decreased efficiency of feed utilization observed in this study as the RBDG inclusion levels increased was in agreement with the findings of Nelson (1984) who observed that increased BDG levels above 30% led to increased feed intake and feed to gain ratio. Dairo (1999) also observed a significant increase in feed conversion ratio as level of sorghum distillers' waste inclusion increased. However, this did not agree with the findings of Yaakugh *et al.,* (1994) who reported no significant difference in feed to gain ratio when BDG replaced 15, 30 and 45% maize in the diets of pigs.

The recorded high mortality in this study was as a result of a strong rainstorm which blew off part of the roof of the experimental house by the 4^{th} week of the trial. The birds were subsequently subjected to a prolonged cold stress due to inadequate heat supply from lanterns and charcoal. Out of the 22.1% overall mortality recorded in this study, 19.2% occurred during the brooding period.

The final live weight of birds on 10% RBDG diet was similar to those on the control diet. This suggests that the inclusion level of RBDG in a broiler diet at 10% will support adequate live weight gain. This result is in agreement with Delic *et al* (1977) who reported satisfactory result in live weight gain of pigs

when 10% BDG was included in growing pigs' diet. RBDG inclusion in the diets did not have any adverse effect on the internal organs of birds. This observation is contrary to the findings of Kandra *et al.*, (1974) who observed that high fibre diets induced significant increase in the weight and size of the various sections of the digestive system in birds. There was no effect of the diets on intestinal length of birds across the different treatment groups. This is in agreement with Dogari (1978) who observed a non-significant effect on intestinal length when maize was replaced at different levels by sorghum beer residue in broiler chick diets.

4.2.0 Effects of Lysine, methionine and enzyme supplementations on the performance and carcass characteristics of broiler chickens fed 30% RBDG diet.

5.2.1 Starter phase (0-4 weeks)

The slight increases in final and daily gains observed on birds on the amino acid supplemented diets over the unsupplemented diet were in agreement with Nnaemeka (1990) who observed better weight gains when lysine was supplemented alone in cotton seed meal diets for broilers, layers and turkeys. Wylie et al (2003) on the other hand observed increased body and breast muscle weights on amino acid (arginine, methionine and tyrosine) supplementation of turkey diets. Nwokoro and Tewe (1998) reported a significant increase in daily weight gains with increase in the dietary methionine levels. The slightly higher

body weight gain of birds on the enzyme–supplemented diet compared to those on unsupplemented diets observed in this study is in agreement with Ravindran *et al.* (2003) who reported improved weight gain of broilers fed maize and wheat based diets supplemented with enzymes (Allzyme SSF)

The significantly higher daily weight gains observed for birds on amino acids plus enzyme supplemented diet could be attributed to the effect of excess methionine and lysine in diet as well as the availability of nutrients from the breakdown of RBDG by the microbial enzyme (Allzyme) as observed by Ohta and Ishibashi(2000).

The similarity in feed conversion observed in birds on the different RBDG diets during the starter phase signifies the effectiveness of all the treatments to maintain adequate weight gain. This is contrary to the findings of Maikano (2005) who reported that rice offal could be fed to broilers from 0-5 weeks at level up to 15% without any adverse effect on growth performance. Nduaka (2006) observed a significantly poorer feed to gain ratio when the un-supplemented 7.5% rice offal diet was compared with the control diet. The significantly higher feed conversion of birds on unsupplemented 30% RBDG diet compared to those on control and those on +LME diet shows the superiority of these diets to effectively support better performance in broiler at the starter stage.

The feed cost per kg gain for + LME diet and for the control diet were similar but significantly cheaper than other diets. This is as a result of the higher final and daily weight gain realized with birds on these diets. The similarity in feed cost for birds on other diets does not agree with the findings of Maikano (2005) who observed no significant effect of diets on feed cost when 15% dietary level of rice offal was fed to broilers with or without enzyme supplementation.

5.2.3 Finisher period (6-9 weeks)

The significant improvement in weight gain and feed efficiency observed with birds on lysine supplementation over the unsupplemented 30% RBDG diet during the finisher phase was in agreement with the findings of Lemme and Petri (2002) who observed improved weight gain and feed efficiency with increasing dietary lysine content for poultry. Nnaemaka (1980) also recorded better weight gains in poultry when dietary cotton seed cake meal was supplemented with lysine alone. Higher weight gain was observed with birds on methionine supplemented diet compared to those on unsupplemented 30% RBDG diet. This result agreed with the findings of Fanimo *et al.*, (1996) who observed that amino acid supplementation brought about improvement in dietary protein utilization. Fanimo *et al.*, (1998) however, did not observe any improvement in the utilization of dietary protein when shrimp waste meal was supplemented with synthetic amino acids (methionine and lysine). The

significantly higher growth rate observed for birds on enzyme – supplemented diet over that of the unsupplemented 30% RBDG diet, revealed the better quality of the diets.

The significantly higher weight gains recorded for birds on + LME diet over those on unsupplemented diet was as a result of higher quality and improved palatability of + LME diet. This resulted in higher feed intake and better feed conversion of birds on +LME diet compared to those on unsupplemented diet. The superior feed intake for birds on + LME diet over other diets could also be as a result of the improved palatability of + LME diet.

The similarity in feed to gain ratio of birds on the control, + lys, + Met, + Enz and + LME diets revealed the effectiveness of these diets to support better weight gains and performance in broiler finishers. The highest feed to gain ratio observed with birds on unsupplemented diet compared to other diets revealed the reason for the lowest average gain observed by birds on unsupplemented diet compared to those on other diets.

5.2.4 Carcass characteristics of finished broilers

The dressing percentage in this study was not affected by dietary treatments. Nwokoro and Tewe (1998) however observed a significant difference in dressing percentage with amino acid supplementation. The percentage breast of birds was observed to be depressed by amino acid and enzyme supplemention

of RBGD diets (+Lys, +Met, +Enz and +LME) when compared to the control and 30% RBDG diet. This finding is in conflict with that of Wylie et al (2003) and Lemme and Petri (2002) who both observed increased breast percentage when a wheat meal based basal diet were supplemented with amino acids (lysine and methionine). Birds on diet supplemented with lysine had significantly lower percentage thigh compared to those on unsupplemented, + Met, + LME and control diets. This observation is in agreement with the findings of Lemme and Petri (2002) who also reported decreased percentage thigh with higher dietary levels of lysine in wheat meal diets.

The percentage liver for birds on the diets supplemented with lysine, methionine and enzyme as well as those on control were similar but significantly higher than those birds fed unsupplemented 30% RBDG diet. This is in agreement with Fanimo *et al* (1998) who observed an increase in liver weight with increased level of amino acid and it is contrary to the report of Brenes *et al* (1993) and Atteh (2004) who observed a decreased liver percentage. The percentage liver on the diet containing lysine, methionine and enzyme was significantly the least compared to percentage liver of birds on other treatments. The lesser the activities of the liver the smaller the expected size and vice-versa. The lower size of the liver on this diet may be an indication of the higher quality of the diet over other diets.

In this study, the percentage gizzard, heart and abdominal fat and the intestinal lengths were also affected by the different dietary treatments. Fanimo *et al* (1998) however, reported similarity in both gizzard weight and intestinal length when broilers were fed shrimp waste meal supplemented with synthetic amino acid. The higher percentage gizzards observed with birds on all diets except +LME diet) compared to the control could be as a result of RBDG inclusion which causes the gizzard to adjust in size to handle the higher fibre content of the diet (Okonkwo *et al.* 1995).

The higher abdominal fat observed in the birds on +LME diet is in agreement with Fanimo *et al (*1998), while the lower abdominal fat observed on + lys and + Met diets is in agreement with Fanimo *et al* (1998). The researchers observed an increase in the abdominal body fat with amino acid supplementation of poultry diets. The intestinal length on enzyme supplemented diet (+Enz) was similar to those on lysine supplemented diets but significantly higher than those on other diets. This is contrary to the findings of Marcquardt *et al* (1996) who reported that enzyme supplementation reduced the length and sizes of various sections of the gastro intestinal tract.

5.3.0 Metabolism of nutrients in RDBG diets supplemented with synthetic amino acids and enzyme.

The enhanced and similar apparent metabolizable DM of the amino acids and enzyme supplemented diets which were compared to the control and higher than the un-supplemented diet was as a result of the supplemental amino acids and the enzymatic degradation of non-starch polysaccharides by the exogenous microbial enzyme (Iji *et al.* 2001). The lowest nutrient metabolism observed for birds on the unsupplemented 30% RBDG diet is as a result of the non-starch polysaccharides in the high fibre diet which does not succumb to enzymes of animal origin (Iji *et al.* 2001), making the diet less metabolized by the bird's enzymes. The significantly higher quantity of ash metabolized by birds fed amino acid and enzyme supplemented diets could be as a result of the enhanced enzymatic activities in these birds. Ishibashi and Ohta (2000) observed that phytase supplementation improved the utilization of chelated minerals and proteins.

The daily ingestion and digestion of ether extract in this study was observed to be similar on all diets except the amino acids and enzyme supplemented diets which were significantly higher for both. The high intake and metabolism of EE of the +LME diet was positively influenced by the amino acids and enzyme supplementation in the diet (Lemme and Petri, 2002).

The apparent CF metabolism by birds on lysine, enzyme and amino acids plus enzyme supplemented diets did not differ. They were also similar to those on

the control but were significantly lower compared with those on the 30% RBDG plus methionine supplemented diets. The lower metabolized unsupplemented 30% RBDG diet was as a result of the high fibre content of the diet, which renders the nutrients unavailable.

The apparent crude protein metabolism of the unsupplemented diet was significantly lower (though similar to that of methionine supplemented diet) compared to that of lysine, enzyme, and +LME and the control diet. This could be explained in terms of amino acid availability from the respective diets. It was established that the dietary amino acid imbalance or availability often results in poor assimilation of the amino acids. This is often noticed in high levels of nitrogen voided in faces and urine (Yaakugh, 1994). It is therefore possible that the Nitrogen utilization from the unsupplemented diet was poor. The digested energy and apparent metabolizable energy of all diets were similar. This shows that both amino acids (+ lys, + Met, and + LME) and enzyme (Allzyme) supplementation does not bring any significant improvement in the dietary apparent metabolizable energy content of the diets over the control and un-supplemented diets.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary of the major findings

6.1.1 Nutrient composition

1 BDG Samples on 2, 4, 6 and 8-day fermentation with 10:1, 10:2, 10:3, 10:4 and 10:5, substrate to inoculum concentrations generally had their CF content reduced and their CP content increased.

2 The BDG sample on 2-day fermentation with 10:2 inoculum concentration gave the highest crude protein content.

3 RBDG samples with 8-day fermentation and 10:5 substrate to inoculum concentration showed the lowest crude fibre content.

6.1.2 Effect of feeding graded levels of RBDG on the performance and carcass characteristics of broiler chickens.

1 Daily feed intake of birds on all RBDG diets increased with increasing levels of RBDG in the diet during the starter and finisher phases.

2 The average daily gain of birds on RBDG diets was lower than those on the control diets during the starter and finisher phases.

3 The feed to gain ratio increased with increasing dietary level of RBDG during both starter and finisher phases.

6.1.3 Effect of feeding 30% RBDG diet supplemented with lysine or methionine or enzyme (Allzyme SSF) and the combination of the three on the performance of broilers.

1 Supplementation of 30% RBDG with lysine alone, with methionine alone, with enzyme (Allzyme) alone, or with the combination of the three improved average daily gain of the birds during the starter phase.

2 During the finisher phase, 30% RBDG diet supplemented with lysine alone or with a combination of lysine, methionine and Allzyme, improved average daily gain and feed conversion of broilers up to the level obtained with the control diet and significantly beyond the level obtained with the un-supplemented 30% RBDG diet.

3 Feed cost was not reduced by any of the supplements during both starter and finisher phases.

4 The 30% RBDG diet whether supplemented or un-supplemented with amino acids and enzyme resulted in increased feed intake above the control diet.

5 The final live weight of birds on +lys, + Met., + Enz and 30% RBDG were similar but lower than those on +LME and control.

6 Abdominal fat of birds on + LME was higher than those on other diets.

6.1.4 Nutrients metabolism in broiler chickens fed 30% RBDG with and without amino acids and enzyme supplementation.

1 Dry matter metabolism of the 30% RBDG diet was improved by the addition of lysine, allzyme or a combination of lysine, methionine and allzyme.

2 Metabolism of ash was improved by the addition of lysine, Allzyme and a combination of lysine plus methionine plus Allzyme.

3 Ether extract metabolism was improved by supplementing the diet with either lysine, Allzyme or a combination of lysine plus methionine plus enzyme.

4 Crude fibre metabolism was improved by supplementing the diet with lysine, enzyme or a combination of lysine, methionine and enzyme.

5 Apparent crude protein metabolism was improved by supplementing the diet with lysine, enzyme or a combination of lysine, methionine and enzyme.

6 Apparent metabolizable energy was improved by supplementing the diet with either lysine, allzyme or a combination of lysine, methionine and enzyme.

6.2 CONCLUSION

From the studies, the following conclusions were arrived at;

1. The fermentation of BDG between the periods of 2-8 days with rumen liquor as inoculum at concentrations of 10:1 to 10:5 resulted in increased percentage crude protein and decreased percentage crude fibre levels.
2. The best substrate to inoculum concentration for BDG refermentation among the test concentrations for the highest %CP content is 10:2
3. The optimum period of re-fermenting BDG for highest %CP content among the test periods is 2days.
4. The percentage DM of BDG samples with concentrations 10:1, 10:2, 10:3, 10:4 and 10:5 fermented for 2, 4 and 8days were unaffected by fermentation.
5. The use of RBDG in broiler starter and finisher diets requires supplementation of the diets with lysine, enzyme or the combination of the three for its effective utilization in the feeds.
6. Supplementation of 30% RBDG broiler starter and finisher diets with lysine, enzyme or the combination of lysine, methionine and enzyme (Allzyme) enhanced metabolism of DM, ash, EE and CF. It also improved apparent crude protein and apparent gross energy

metabolism.

8 Birds on combined amino acids and enzyme supplemented diet exhibited superior performance over those on un-supplemented diet (30% RBDG) during both starter and finisher periods.

7. Supplementing 30% RBDG diet with lysine, methionine and enzyme resulted in greater feed intake.
8. 30% RBDG broiler diet supplemented with a combination of lysine, methionine and enzyme (+LME) resulted in greater daily gain at both starter and finisher periods.

6.6 Recommendations of the study

From the findings in these studies, the following are recommended;

1. Two (2) day commercial fermentation of BDG with rumen liquor as inoculum at concentration of 10:2 for the enhancement of its nutritive value for utilization in monogastric nutrition

2 Adequate supplementation of refermented BDG with amino acids and or enzymes for use in broiler rations as a way of improving the performance.

3. The use of refermented BDG in diets of monogastrics as a means of reducing pressure on conventional protein sources like GNC and soya bean.

6.7 Recommendations for further studies

For further studies, the following recommend;

1. Further studies into the replacement of different proportions (0, 25, 50 or 75%) of the major protein source in diets of broilers..
2. The evaluation of the effect of feeding RBDG diet supplemented with lysine-enzyme (+LE) and methionine-enzyme (+ME) combinations on the performance of broiler chickens.

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APPENDIX

The RBDG sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and then loaded into the Technicon sequential multi-sample Amino Acid Analyzer (TSM) for 76 minutes.

Calculation of amino acid values from the chromatographic peak:

The net height of each peak produced by the chart record of the TSM (each representing an amino acid) were measured (figure 2.3), the half height of the peak on the chart was found and the width of the peak at the half height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half height. The norleucine (NE) for each amino acid in the standard mixture was calculated.

The TSM linear chart record showing the chromatograph peaks charts record for the standard mixture (std) amino acid profile is a shown in figure 2.2, while the chromatograph ic peak chart of the amino acid analyzed in RBDG is as shown in fig 2.3

From the following figures, the concentration of each amino acid in RBDG was calculated after the net weight and width at half height of each of the

measured chromatograph peaks;

Weight of sample hydrolyzed = 0.3205

N_2 (fat free = 6.24

Volume loaded (basic AA) = 10L

Volume loaded (Acidic /Neutral AA) = 5L

Dilution = 5

The results of the measurement carried out and concentration of each amino acid obtained by calculation were as shown in table 3.2.