BIOCONVERSION OF UREA AND METHIONINE SUPPLEMENTED SORGHUM BEER RESIDUE INTO PROTEIN BIOMASS FOR BROILER RATIONS

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DECLARATION

I declare that the work in this thesis entitled "**Bioconversion of ureaand methionine supplemented sorghum beer residue into protein biomass for broiler rations** has been performed by me in the Department of Animal Science under the supervision of Professor (Mrs) A.A Sekoni, Professor S. Duru and Dr. T. S. Olugbemi.

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

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NAME OF STUDENT **SIGNATURE** **DATE**

CERTIFICATION

This thesis entitled "BIOCONVERSION OF UREA AND METHIONINE SUPPLEMENTED SORGHUM BEER RESIDUE INTO PROTEIN BIOMASS FOR BROILER RATIONS" by Ndams Samuel Shehumeets the regulation governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this worksolely to God Almighty. My Savior and Wisdom.

ABSTRACT

In a laboratory study aimed at determining the effect on nutrient composition of sorghum beer residue (SBR) supplemented with urea and methionine and passed through a 2nd fermentation process. Four (4) levels of urea (0.5,1.0,1.5 and 2.0%) and four (4) levels of methionine (0.29, 0.33, 0.37, and 0.41%)were mixed which resulted in a total of sixteen (16) urea to methionine mixing ratios. Sixteen (16) samples of SBR were treated with the 16 urea and methionine ratios and subjected to 4 days fermentation with bovine rumen liquor as inoculum. SBRsupplementation with urea to methionine ratio of 1.5: 0.37 % after 4 days fermentation resulted in significant increase in protein biomass from 21.73 to 38.19%. The protein biomass produced contained all the essential amino acids which were superior compared to those of groundnut cake (GNC) and other feed ingredients.In an experimental trial, the effect of feeding urea and methionine supplemented and refermented SBR (RSBR) at 0, 25, 50, 75 and 100% replacement levels in broiler chickens starter and finisher diets were investigated. Five dietary treatments were formulated to be iso-nitrogenous and iso-caloric to provide 23 and 20.5% crude protein (CP) for starter and finisher diets respectively. Three hundred and forty-five (345) day-old broiler chicks (Zam breed) were randomly allocated to 15 pens in groups of 23 birds per pen. The five diets (replicated three times) were randomly allocated to the pens of broilers on deep litter concrete flooring.(At the end of starter and finisher phases, daily feed intake (DFI), weight (WG) gain, feed: gain (F/G) ratio and feed cost per kilogram (kg) of body weight gain were evaluated). Daily feed intake of broiler chickens on 50 and 75% RSBR diets were similar ($P>0.05$) and significantly higher ($P<0.05$) than those on other diets during the starter phase but during the finisher phase, DFI of birds on 50 and 100% RSBR were similar ($P>0.05$) and significantly different ($P<0.05$) compared to those on control and other diets. Daily weight gain (DWG) of birds on 25, 50 and 75% RSBR were as good ($P>0.05$) as the control diet at starter period. The daily weight gain of birds on the control, 25 and 50% RSBR diets were similar ($P>0.05$) but better than that of birds on 75 and 100% RSBR diets during the finisher period. Feed to gain ratio of birds fed the control and 25% RSBR inclusion levels were similar ($P>0.05$) and better ($P<0.05$) than those fed on other diets during the starter and finisher periods respectively. Feed cost per kg gain of body weight of birds on 25 and 50% RSBR starter and finisher diets were similar to the control diet, and significantly lower ($P<0.05$) compared to that of birds on 75 and 100% RSBR. Mortality of birds during the eight-week trial on the treatments was low. Dressing percentage of birds on 50% RSBR diet was significantly higher ($P < 0.05$) than those on control and other diets. Heart and gizzard percentages of birds on 100% RSBR diet were significantly higher ($P < 0.05$) than those on control and other diets.The parked cell volume, hemoglobin and total protein concentrations (g/dl) were within normal values.This study indicates that economically, up to 50% of RSBR could replace GNC in the diets of starter and finisher broiler birds respectively without adverse effect on growth performance and carcass yield.In a second feeding trial, performance and carcass characteristics of broiler chickens fed 75% urea and methionine treated and fermented sorghum beer residue (RSBR) diets supplemented with and

without enzymes (Nutriad®(N), Zymos-N® (Z) and Fullzyme® (F)) were investigated. Three hundred and forty five (345) day-old broiler chicks were randomly assign to five (5) dietary treatments with three (3) replicates (15 pens), each consisting of twenty three (23) birds in a completely randomized design. Diets 1 (control), 2, 3, 4, and 5 contained 0% RSBR, 75% RSBR, 75% RSBR plus Nutriad (+ N), 75% RSBR plus Zymos-N(+Z) and 75% RSBR plus Fullzyme(+F) respectively. The daily feed intake and weight gain of birds on +N and +Z diets were similar (P>0.05) but significantly higher (P<0.05) than those on control, 2 and 5 (+F) diets during the starter and finisher periods. The feed to weight gain ratio during the starter period were similar (P>0.05) for birds on all enzymes supplemented diets but significantly lower (P<0.05) compared to birds on control. During the finisher phase, the feed to gain ratio was least (P<.0.05) with birds on +N diet and highest (P<.0.05) with birds on 75% RSBR diet. While feed cost per kg gain was significantly lower (P<0.05) during the starter phase for birds on +F.The least feed cost per kg gain during the finisher period was shown by birds on +N diet compared to that of birds on other diets. The dressing and thigh weight percentages were significantly higher (P<0.05) for birds on +N diet compared to those on control and other diets. The heart and gizzard percentage for birds on 75% RSBR were significantly higher (P<.0.05) compared to that for birds on other diets. This study indicated that feeding 75% RSBR supplemented with Nutriad and Zymos-N enzymes supported growth performance and carcass yield at lower feed cost per kilogram of broiler chicken weight produced.

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List of Abbreviations

ABU	-	Ahmadu Bello University
ADF	-	Acid Detergent Fibre
ADG	-	Average Daily Gain
AME	-	Apparent Metabolisable Energy
A.O.A.C.	-	Association of Official Analytical Chemists
BDG	-	Brewers Dried Grains
Cyst.	-	Cystine
CSFC	-	Community Food Security Coalition
CF	-	Crude Fibre
Cfu	-	Colony Forming Unit
CP	-	Crude Protein
CPH	-	Cocoa Pod Husk
C/N	-	Carbon to Nitrogen ratio
DAF	-	Diammonium Phosphate
DFI	-	Daily Feed Intake
DMD	-	Dry Matter Digestibility

DM	-	Dry Matter
DMI	-	Dry Matter Intake
EE	-	Ether Extract
FAN	-	Free Ammonia Nitrogen
FSBRN	-	Fermented Sorghum Beer Residue Nitrogen supplemented
FSBRS	-	Fermented Sorghum Beer Residue Sulphur supplemented
FSG	-	Food Security Guide
FAO	-	Food and Agricultural Organization
FSC	-	Food Security Centre
F/G	-	Feed to Gain Ratio
+F	-	Plus Fullzyme
GE	-	Gross Energy
g/dl	-	Gram Per Decilitre
GNC	-	Groundnut Cake
Hb	-	Haemoglobin
KG	-	Kilogram
KNARDA	-	Kano State Agricultural Development Authority

ME	-	Metabolizable Energy
MDG	-	Millennium Development Goals
Met.	-	Methionine
NDF	-	Neutral Detergent Fibre
NFE	-	Nitrogen Free Extract
NPN	-	Non Protein Nitrogen
NSP	-	Non Starch Poly-saccharides
NRC	-	National Research Council
N/S	-	Nitrogen, Sulphur ratio
+N	-	Plus Nutriad
PCA	-	Principal Component Analysis
PCG	-	Polymer Coated Grade
PCU	-	Polymer Coated Urea
PCV	-	Parked Cell Volume
RSBR	-	Refermented Sorghum Beer Residue (Urea and methionine supplemented)
SBR	-	Sorghum Beer Residue
SDW	-	Sorghum Distillers Waste

SSF	-	Solid State Fermentation
SEM	-	Standard Error of Mean
SCP	-	Single Cell Protein
TP	-	Total Protein
TND	-	True Nitrogen digestibility
TCPH	-	Treated Cocoa Pod Husk
VSC	-	Volatile Sulphur Compounds
VFA	-	Volatile Fatty Acids
YAN	-	Yeast Available Nitrogen
W/W	-	Weight by Weight
+Z	-	Plus Zymos-N

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background and statement of the problem

According to Food Security Guide (FSG) (2011), over one billion people experience the hardship that hunger imposes. Today these figures continue to rise even amidst the riches of the 21st century. With the increasing population growth, economic instability and climate change, today food security has become an urgent challenge for national and global governance.

When the FAO Cereal Index doubled in 2008 according to Community Food Security Coalition (CFSC) (2011), food security became a global crisis. That the world's poorest household spends 60-100% of their income on food; without any mechanism to cope with rising prices other than to reduce the volume or nutritional quality of their consumption. At the 2009 World Summit on food security, richer countries were called to reorganize their misplaced priorities in order to tackle global food insecurity. Despite many similar political commitments, the number of people lacking access to minimum diet rose from 824 million in the Millennium Development Goals (MDG) baseline year 1990 to 1,020 million in 2009 (CFSC, 2011).

A 2009 report by the International Food Policy Research Institute estimated that in the absence of resolute government action, food availability in the sub-Saharan African region will average 500 calories less per person in 2050, a 21 percent decline (CFSC, 2011). The Food Security Centre (FSC) at the University of Hohenheim, Stuttgart, Germany, had a mission to make effective and

innovative scientific contributions in research, teaching and policy advice to eradicate hunger and achieve food security in Africa, Asia and Latin America (FSC, 2011).

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. Beszedits and Lugowski as cited by Shier and Purwono (1994) reported an estimated 4 million metric tons annual shortfall of protein worldwide.

This trend in protein shortfall 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 was still a far cry from the recommended 54g 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 chicken production cannot be over emphasized.

According to FAO (1984), when feed resources are limited, the best approach is to utilized crop residues and agro-industrial by-products more effectively. The global shortage of food and feed proteins has prompted animal nutritionist to seek protein production improvements from both conventional and nonconventional sources. One non-conventional source is the mass cultivation of microbial biomass using renewable substrates which occur abundantly in nature. 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.

Ruminant nutritionists and microbiologists have long been interested in manipulating rumen fermentation 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. The organisms use nitrogen, phosphorus, and other nutrients in developing cell protoplasm. A typical count of bacteria number in the rumen according to Church and Pond (1988) approaches between 25-50 billion/ml while typical counts of protozoa are in the order of 20-50,000/ml. 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).

In the fermentation process, waste products are transformed into new ingredients for use as feeds (Kherrati *et al.,* 1998). The most commonly used substrates in solid state fermentation (SSF) are products of agro-residue which include bran, straw, sugarcane bagasse and residues of fruit processing industries (Suffian *et al*., 2010). SSF has been employed in the production of animal feed, hydrolytic enzymes, organic acids, gibberellins, flavors, bio pesticides, ethanol and mushrooms (Suffian *et al*., 2010).

Another by-product of considerable importance is the dried sorghum beer residue (SBR), which is a residue of home brewed beer in traditional Africa. It is a residue arising from the local brewing of “Burukutu” and “Pito” in Nigeria (Tyokpat, 1989), “Atella” in Ethiopia (Demeke, 2007) and “Dolo” in

Burkina Faso (Pousga *et al*., 2007). There is growing interest in the use of SBR in Africa for livestock nutrition. The proximate composition of brewers' sorghum beer residue was reported by Abdulmalik (1998) as follows: 93.72% DM, 30.16% CP, 11.82% EE, 12.93% CF, 2.51% Ash, and 42.58% NFE. Levels up to 20% and 15% of SBR were recommended by the author for inclusion in the diets of weaned and breeding does respectively. Pousga *et al.* (2007) gave the chemical composition of sorghum beer residue in Sapone area of Burkina Faso as follows; 94.79% DM, 23.3% CP, 7.9% CF, 5.5% EE, 7.5% Ash, 40.1% NDF, and 20.7% ADF. In Ethiopia, Demeke (2007) reported the chemical composition of "Atella" as follows: 92.3% DM, 21.8% CP, 7.15% EE, 54.0% NDF, 29.0% ADF and 5.80% Ash. The inclusion of "Atella" as the sole source of protein in starters ration of broilers chickens significantly ($P<0.05$) lowered feed intake and weight gain (Demeke, 2007).The local breweries for "Burukutu" and "Pito" are scattered all over Nigeria especially in parts of Kaduna, Benue, Bauchi, Borno and Gongola states (Tyokpat, 1989). SBR is therefore much accessible in these areas of the country.

With this insight and the quest for sourcing cheap feed ingredients, this study is aimed at enhancing the nutritive value of SBR with respect to protein content and quality through solid state fermentation after pretreatment with nitrogen and sulfur using rumen liquor as inoculums, as well as accessing the ideal level of incorporation into broiler diets.

1.2 Justification of the study

This study was motivated by the fact that large quantities of sorghum beer residue are being produced in Nigeria by the local brewing industry all year round that can be recycled

for monogastric nutrition. The use of fermentation processes to produce microbial biomass has several advantages over other unconventional processes that rely on agricultural by-products, including the fact that fermentation processes are not subject to the variability of weather conditions and they can be controlled for product quantity and quality in virtually any geographic location (Rudravaram *et al.*, 2006). Microbiological biosynthesis of protein has been reported to exhibit a fairly high growth rate, high protein biomass, high protein digestibility and interesting amino acid composition (Oshoma and Ikenebomeh, 2005). The process will not only help to recycle the sorghum beer residue but will also help to clean the waste. This process is also believed to be devoid of ethical and animal rights issues (Shahera and Ashour, 2002).

The solid state fermentation (SSF) offers numerous advantages for the production of proteins because of its high productivity, low-cost media, and less effort in processing (Yalemtesfa *et al*, 2010). The process will be cheap and simple to produce and is without negative effect on the environment. The technology can easily be adopted by both small and large scale farmers. This study will extend existing knowledge on the nutrient composition and utilization of sorghum beer residue in broiler chicken production. Also, some methodological steps to be taken in answering every question raised will be of great significance to professional peers.

1.3 Objectives

The objectives of the study were to determine;

i. The effect on nutrient composition of sorghum beer residue passed through a 2nd stage fermentation process.

ii. The effect on nutrient composition of SBR with urea, methionine, and with urea and methionine supplementation passed through a 2nd stage fermentation process.

iii. The performance of broiler chickens raised on urea and methionine supplemented and refermented Sorghum Beer Residue (RSBR) based diets.

iv. The effect of urea and methionine supplemented and fermented SBR diets on carcass characteristics and organ weights of broiler chickens.

v. The performance of broiler chickens fed urea and methionine treated and fermented SBR, supplemented with Nutriad, Zymos-N and Fullzyme multi-enzymes using the best diet of objective iii above.

vi. The haematological characteristics of broiler chickens fed RSBR based diets.

vii. The effect of RSBR diets, supplemented with multi-enzymes on carcass characteristics and organs weights of broiler chickens.

viii. The effect of utilizing enzymes supplemented RSBR on the economic cost of producing a kilogramme weight of broiler chickens.

1.4 **Research Hypothesis**

The research hypothesis for this study were;

i. There are differences in nutrient composition of SBR when passed through a 2nd stage fermentation.

ii. There are differences in nutrient composition of SBR with urea, sulfur, and with urea plus sulfur supplementation when passed through 2nd stage fermentation.

iii. There are differences in performance of broiler chickens raised on RSBR based diets.

iv. There are differences in carcass characteristics and organ weights of broiler chickens raised on RSBR based diets

v. There are differences in haematological characteristics of broiler chickens raised on RSBR based diets.

vi. There are differences in performance of broiler chickens raised on RSBR diets supplemented with multi-enzymes.

vii. There are differences in carcass characteristics and organ weights of broiler chickens raised on RSBR diets supplemented with multi-enzymes.

viii. There are differences in terms of economic cost of producing a kilogram weight of broiler chickens raised on RSBR based diets

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Renewable biological materials as biomass

The World's most abundant and readily accessible natural waste product is available in the form of plant tissue called biomass. Habu (2011), referred to all forms of plant-derived matter (terrestrial and aquatic) other than the fossilized as biomass. Biomass is the organic matter in trees, agricultural crops and other living plant materials, making up of among others, carbohydrates, proteins and organic compounds that are formed throughout the growing plant life period. He enumerated the various forms of biomass resources in the world to include; wood residues (from

wood product industry which includes paper mills, saw mills and furniture manufacturing), municipal solid waste and agricultural crops and residues.

Biomass as earlier defined by Habu (2011) were derived from three sources, namely: forestry and wood processing residues, crop residues and animal waste (Monique *et al*., 2003). Agricultural and forest residues are the main sources of biomass available for conversion to energy. They are abundant, comprising of about 80% of the world's biomass (Habu, 2011) and are outside the human food chain making them relatively inexpensive feedstuffs for livestock nutrition. The various categories of plant biomass according to him are; woody biomass, non-woody biomass, processed waste and fuels from plant biomass. Straka *et al*. (2007), classified natural bio-degradable biomasses into three main groups as saccharides (sugars, starch, cellulose and hemicellulose), fats (lipids) and proteins. Among the 3-groups of biomass only proteins are carriers of sulphur and nitrogen.

2.1.1 Woody Biomass

The woody biomass is present naturally in forests and woodland and includes the trees, shrubs and scrub, forest floor litter, palms and bamboo, amongst others. According to Monique *et al.*(2003), woody biomass are utilized in the production of bio-energy and the full range of bio based products including lumber, composites, paper and pulp, furniture, housing components, round wood, ethanol and other liquids, chemicals, and energy feed stocks. They observed the beneficial effects of biomass utilization in social, ecological and economical terms.

2.1.1.1 Social Benefits

As social benefits, woody biomass reduce the threat and impact of wildfires on communities, improve recreation/scenic opportunities by thinning overcrowded forests, improve human health through better air quality and reduced wildfire and prescribed fires emissions, provide rural community vitality though the provision of sustainable environments and economies over the long term, provide increase societal awareness by using forest restoration activities as a learning tool to promote wise forest management and lower treatment costs by finding new markets for removed residue (Monique *et al*., 2003).

2.1.1.2 Ecological and Environmental Benefits

These include, decrease insect and disease outbreaks toward endemic levels, decrease unnaturally severe wild land fires within forests and grasslands, facilitate the removal of invasive woody species, increase ability to protect and restore critical wildlife habitat, provide clean air through decreased wildfires size and severity, increase the longevity of landfills which reduces the amount of land that needed to be converted into new landfills, Improved vigor of remaining trees, reduce fire related erosion and maintain healthy watersheds, improved forest health, reduced dependence on fossil fuels, reduced greenhouse gas emissions and deduce atmospheric concentrations of greenhouse gases through substitution of fossil fuels and provision of “carbon neutral” energy when woody biomass is regrown.

2.1.1.3 Economic Benefits

Generally, woody biomass can be exploited for different applications as in synthesis of chemicals and pharmaceuticals, production of animal feeds or food and manufacture of construction materials. The use of raw biomass is applicable in extracted fibres and other compounds to produce

starch-based plastics, building materials (e.g wood products or strand board), pulps and papers, yarns, fibres and fabrics, fertilizers, soil improvers and compost (Habu,2011) Some other economic benefits of woody biomass utilization include:

- Reduction in the cost of hazardous fuels treatments, which indirectly reduces wildfires. Wildfire prevention enhances or preserves wildlife habitat and watersheds, avoids smoke and fire-related health and safety issues, protects property and recreational values, and avoids the costs of fire suppression and rehabilitation.
- Reduction in the cost of healthy forest and range management by providing a market for insect or disease-infested trees, invasive species, and other woody biomass removed.
- Lowers greenhouse gas emissions over fossil fuels, because the carbon dioxide released when woody biomass is burned is balanced out by new, carbon-sequesteringbiomass growing in its place. Durable wood products from biomass continue to store carbon absorbed by trees when they were growing.
- Provision of employment and economic stability to rural, forest-dependent communities, investments attraction in new industries and markets and stabilize existing markets including tourism.
- Complementation of traditional utilization of higher values wood products, (Schneider and McCar, 2003).

2.1.2 Non-woody Biomass

The non-woody biomass refers to those that can be grown specifically as a primary source of fuel for conversion to motor alcohol (ethanol). This primary source of fuel is known as the energy crops. These include crops like sugar cane, cassava, potatoes, tobacco stems and roots, banana, plantain, swamp and water plants, switch grass, cereal straw, and pulses, etc. othernon-woody biomass fuels

like straw, manure and sludge are cheap fuels with an increased chlorine, sulphur and ash contents. The replacement of expensive woody biomass fuels with non-woody biomass has a high economic potential, due to the low price of the non woody biomass (Schneider and McCar, 2003).

The electricity costs can potentially be reduced by the application of cheap biomass fuels like manure, sludge, and straw. However these cheap non-woody biomass fuels often contain a considerable amount of ash, chlorine and sulphur. The application of non-woody cheap biomass fuels like straw, manure and sludge in gasifies for the production of electricity and heat seems to be economically attractive. Non-woody biomass is in this report waste from the industry, agriculture and animals. Examples of non woody biomass are grass, straw, manure, and sludge. An important difference between woody and non-woody biomass is the relatively high chlorine, sulphur and ash content in non-woody biomass. The relatively high sulphur content could be explained by the relatively high protein content in manure and sludge.

2.1.3 Processed form of plant biomass

These include charcoal (wood and residues), briquettes, and woody alcohol in form of ethanol or methanol, plant oils, bio-diesel/petrol, producer gas and biogas. Charcoal has been used as an antidote for poisoning, for drawing and as fuel in metal works as fire. Ethanol is a clear, colorless liquid with a burning taste and characteristic agreeable odor. Ethanol is the alcohol in beverages such as beer, wine and brandy. It has been used as the fluid in thermometers for temperatures below -40^{o} C, and as an anti-freeze agent in automobile radiators because of its low freezing point (Kim and Dale, 2004).

Petroleum is a naturally occurring oily, bituminous liquid composed of various organic chemicals, found in large quantities below the surface of earth as fuel while biogas a mixture of methane and carbon dioxide produced by decomposing organic matter used as cooking fuel. Bio-diesel is a combination of unleaded gasoline and ethanol made from corn which reduces fossil energy used by 50 to 60% and pollution by 35 to 46% (Kumar *et al*., 2008).

2.1.4 Processed waste

This refers to waste that were processed on an industrial scale for use in other energy intensive processes. According to Howard *et al.* (2003), these include; nut shells and flesh, cereal husk and cobs, bagasse, sisal waste, sawmill waste, municipal waste, plant oil cakes, fruit waste, brewers waste etc.

Biomass is composed of plant fibres that have cellulose as a prominent component. These fibres can be hydrolyzed to yield a variety of sugars that can be fermented by microorganisms. Agricultural residues are of lignocellulosic biomass which are renewable, inexpensive and chiefly unexploited for use (Howard *et al*., 2003)

2.2. 0 The use of agro-industrial by-products in poultry nutrition

Community Food Security Coalition advocated a condition in which all community residents obtain a safe, culturally appropriate, nutritionally sound diet through an economically and environmentally sustainable food system that promotes community self-reliance and social justice (CFSC, 2011).

In this decade, human consumption of meat and eggs of poultry had increased which 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 incorporated in poultry diets to replace the conventionally feed ingredients. More recently, the growth of animal feed industry had allowed considerable use of by- products which havehigh fibre and low energy contents, bulk, low density, low digestibility of amino acids and anti-nutritive factors (Ishibashi and Ohta, 2000). However, the limitations 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. Regardless of the many opportunities already being explored, challenges still exist on costless renewable waste materials with potential of being utilized as substrates for microorganisms to grow upon, and produce useful products for monogastric nutrition. The most prominent by-products of plant origin in use today as enumerated by Ndams (1991) are mainly of oil seed mills, brewing and malting industries.

2.3.0 By-products of brewing and malting industry

The by-products of brewing and allied malting industries are mainly brewers grains, brewers dried yeast, malt sprouts and dried spent hops. 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 de-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 brewer's 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 and Abdulmalik, 1998), "Atella" (Demeke, 2007) and "Dolo" (Pousga *et al.*, 2007).

2.3.1 Brewers' dried grains (BDG)

Bello (1984) and McDonald *etal*. (1998) defined BDG as dried extracted residue of barley malt, alone or in mixture with other cereals resulting from the production 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. The wet product could be dried to about 100g water/kg and sold as dried brewer's grains. Brewers' grains were 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.

2.3.1.1 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/g 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 of 20700 KJ/kg DM for proximate composition of 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, 0.52P% and 2080 kcal ME/kg. 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

2.3.1.2 Effect of brewers' 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 Pascual (1981) evaluated BDG at levels of 0, 10, 20, 30 and 40% in broiler diets and reported no significant difference 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 were used, there were significant reduction 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 no significant differences in egg production parameters up to 25% level. At the 40% level however, egg weights and production rates fell drastically and there were 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 egg production and egg weights. 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.

2.3.2 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 Adamawa States (Dogari, 1985). Inhabitants of the Savannah zone of Nigeria practice the use of the residue in animal feeding, but information on its nutrient contents and prospects of increased utilization is said to be rather scanty (Tyokpat, 1989).

2.3.2.1 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, while 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.

2.3.2.2 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 no significant 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 (McDonald *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 a non-significant 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 *etal.* (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, Paliev *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 was 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.Research has also shown that higher levels of dietary fibre in pigs depressed growth and increased feed intake significantly. Frank *etal.,* (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 monogastric animals. 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

were 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 McDonald *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 fed to uphold high level of meat, eggs or milk production. The commonest physical 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 (Abdullah *et al.,* 2004) to be accomplished through mechanical treatment (grinding), physical treatment (heat), and chemical treatment (caustic alkali, ammonia). 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 feed intake of animals, probably because of increase in the surface area that is in contact with enzymes 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 (McDonald *et al*., 1998).

2.5.1.2 Ionizing radiation

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

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 were 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 according to Walker (1984) is the high-pressure steam method. The author observed that steam treatment processes is expensive 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 cell wall polysaccharides, cellulose and hemicelluloses are hydrolyzed, thereby causing the carbohydrates to become more available to the microorganisms 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 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 in broiler starter diets at varied concentration of 0, 50 100 and 150g/kg did not significantly reduce growth performance and apparent digestibility at levels up to 100g/kg treated CPH 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; McDonald *et al.*, 1998) requires two containers (drums). One for soaking in sodium hydroxide (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 between 0.5-0.7 percent.

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 *etal.* (1998) recommended that 170 litre/tonne of straw of a solution of 300g/l of NaOH. This process was believed to improve digestibility to a slightly less 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 pre-treatment 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 bio-degradation 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 acids. In the study of Wilkinson (1984), he 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 of storage.

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 on wet basis for 42 days. They achieved desirable fermentation in all the silages, 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 silage. 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 *etal.* (1960) soaked chopped straw in an ammonia solution mixed with hay and field peas and stored for 3days. 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 had been reported as a source of ammonia for hydrolysis. Thus KSADA (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. The use of animal urine as a source of ammonia at the rate of one litre per kg of straw stoked above ground for 20days, was observed to increased organic matter digestibility from 45 to 55% (Sundstol, 1981).

2.5.3 Biological treatment

Although mechanical, physical and chemical treatments of straws and agro by-products increases their digestibility, these methods are expensive, require energy input and pollute the atmosphere (Abdullah *etal*., 2004). While Freer and Detroy (1982),attest that biological treatment method is an inexpensive, takes time, but is complete and environment friendly.

Zandrazil (1984) pointed out that the main problem of biological upgrading of ligno cellulose materials into feed was the availability of suitable microorganism with metabolic pattern different from those of the rumen flora and fauna. The authe 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 were 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 *etal*. (1998) reported that rumen content contains 850-930g water/kg on the average and the number of bacteria per ml of rumen content was 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 *etal.*, 1998).

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

Microorganism according to Tegbe (1976), have rapid growth rates and their growth was 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. The problems listed above however were not without solutions. According to the author, the availability of the urease (enzyme) in 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 had been overcome with synthetic methionine supplementation.

Lignocellulose materials that have been studied as substrate for the production of SCP according to Joao *etal.* (1995) included rice straw, corn cobs, sugar bagasse and eucalyptus. By-product feeds that were low in rumen available carbohydrate had reduce microbial protein output (Stokes *etal.*, 1991). 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 *etal.*(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 (1993) fermented fresh cassava peel for 4-days (96hrs) at 30^0C using *Rhizopus spp.* 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 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 increased crude protein and ether extract. Joao et *al.* (1995) fermented Eucalyptus hemi cellulose hydrolysate to produce microbial protein by *Paecilomycesvariotii* (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, corn cob and Eucalyptus (Joao *et al.,* 1995).

2.8.0 Effect of urea and sulphur supplementation on fermentation of feedstuff

2.8.1 Effect of urea supplementation on protein Biomass production

Many fiber-digesting rumen bacteria require ammonia for protein synthesis (NRC, 2001). Urea offers an alternative source of nitrogen for ruminant feeding. Its usage came into

prominence in America due to the shortage of commonly used protein supplements during World War II (Hamilton et al., 1948 as reported by Kononoff *et al.* (2006)). The rumen microbes have a unique role of growing on simple nitrogen containing fractions of feed. Thus, non-protein nitrogen (NPN) containing compound and proteins (N) are used for building microbes which are digested in the abomasum by the host to supply high quality proteins.

Proteins $\xrightarrow{\text{Slow}}$ NH_3 $\xrightarrow{\text{slowly}}$ Microbes

Hamilton *et al*., 1948 as reported by Kononoff *et al*. (2006) found that urea is as satisfactory a source of nitrogen for growing lambs as are most ordinary protein supplements, provided at least 25 percent of the feed nitrogen is in the form of preformed protein and provided further, that the total protein equivalent does not exceed 12 percent. McDonald *et al*. (1998) revealed that the utilization of NPN by ruminants involves its conversion to ammonia and subsequent synthesis into microbial protein.

Urea (NPN) $\xrightarrow{\text{+ Energy (Fast)}}$ NH_3 $\xrightarrow{\text{slow}}$ Microbes

According to the author, the unique ability of the ruminant animal to accomplish this feat provides the mechanism for sparing tremendous amounts of preformed high quality plant and animal protein for utilization in monogastric diets. Urea is commonly added to ruminant diets as a source of non-protein nitrogen that is rapidly hydrolyzed to ammonia in the rumen. Van Soest (1994) stressed that much of the available ammonia was poorly utilized at peak

levels, and that often, the peak level occurs sooner than the peak of maximum fiber fermentation.

Several methods have been evaluated that attempt to improve rumen ammonia by reducing the rate at which urea is released. Such methods include the use of products such as; biuret (Loest *et al*., 2001), starea (Bartley and Deyoe, 1975), and urea formaldehyde (Prokop and Klopfenstein, 1977). Although these methods may be useful in preventing ammonia toxicity, they rarely improve utilization of dietary nitrogen or improve animal performance compared with standard feed sources (Owens and Zinn, 1988). Additionally, Owens and Zinn (1988) revealed that achieving the slow-release effect of some products, such as starea, require heating to bind nitrogen in an insoluble form and may reduce the availability of some nutrients to rumen microbes.

Optigen 1200 (CPG Nutrients, Syracuse, NY) is a polymer-coated, controlled-release urea product (PCU), that compared with feed-grade urea, was believed to possess a reduced rate of ammonia release to rumen microbes (Galo *et al.*, 2003). Hoover and Stokes (1991), proposed the use of PCU in high-forage, low-concentrate diets that have a slow rate of fermentation based on the common understanding that the rate of rumen ammonia production should be paired with that of carbohydrate digestion. Factors which may influence utilization of urea include dietary nitrogen, dietary sulphur, and amount of urea, digestible carbohydrate availability, and length of trial (Hoover and Stokes, 1991).

The use of nitrogen in fermentation is also prominent in wine making. Yeast requires nitrogen for fermentation. If there is not enough, yeast cells are stressed and produce excess

H_2Sthat gives fermentations an off-odor (Anonymous, 2009). According to Cristian *et al*. (2004), lack of nitrogen diminished yeast's metabolic activity, as well as the biomass yield. The H_2S is converted to mercaptans almost immediately, and fermentation without adequate nitrogen will leave one with reduced sulfur compounds and off-smells in the finished wine (Anonymous, 2009).

Yeast available nitrogen (YAN) is a measure of the amount of nitrogen available to the yeast in grape juice measured in parts per million (ppm) or milligrams per liter (mg/L) (Anonymous, 2009). The YAN in grapes according to Ugliano *et al.* (2009) came in two main forms. One was the assimilable nitrogen from the alpha amino acids in the grapes (a-amino nitrogen), and the other is Free Ammonia Nitrogen (FAN). The two sources are important, a-amino nitrogen plays a bigger role at the start of fermentation, while ammonia (FAN) usually supplied the bulk of the nitrogen required during the latter stages of the ferment.

There were no general rules of thumb that helped very much in deciding the amount of additions according to Cristian *et al.* (2004). As a practical rule, everyone agreed that you have to keep your nose in the fermentation to make adjustments as the fermentation proceeded. If pronounced H_2S smell from the ferment is perceived, the yeast is stressed from a lack of nitrogen and had to be corrected immediately. The best thing, however, is to know how much nitrogen to add to the fermentation and add it before problems emerge. The temperature of the fermentation also affects nitrogen requirements. Lower temperature fermentations (as are typical with white wines) require less nitrogen and also usually result in more efficient transformation of sugar to alcohol. For example, Vin-13 is specifically

described by manufacturers as having low nitrogen requirements at low temperatures (Ugliano *et al*., 2009).

According to Ugliano *et al*. (2009), the effect of temperature on fermentation can be ignored when very high fermentation temperature are used. Ugliano *et al.* (2009), supplemented Shiraz (red grape) with low yeast assimilable nitrogen (YAN) with two increasing concentrations of diammonium phosphate (DAP) and fermented with one *Saccharomyces cerevisiae* and one *Saccharomyces bayanus* strain. He observed for the *S. cerevisiae* yeast strain, that addition of DAP to a final YAN of 250 or 400 mg/L resulted in an increased formation of H_2S compared to non-supplemented fermentations (100 mg/L YAN). The *S. bayanus* strain showed a different H_2S production profile, in which production was inversely correlated to initial YAN. For both yeasts, DAP supplementation yielded higher concentrations of organic volatile sulfur compounds (VSCs) in the finished wines, including sulfides, disulfides, mercaptans, and mercaptoesters.

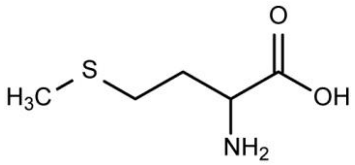
Principal Component Analysis (PCA) indicated that nitrogen supplementation before fermentation determined a much clearer distinction between the VSC profiles of the two yeasts compared to non-supplemented fermentations. Yalemstesfa *et al*. (2010) investigated the production of protein enriched biomass from nitrogen supplemented orange waste as a substrate for the fermentation process using *Aspergillus niger* (KA-06) and *Chaetomium Spp* (KC-06) inoculums. The maximum protein content of 39.64% was obtained with *Chaetomium Spp* at inoculums load of 10^8 spores per ml. *Aspergillus niger* gave a protein yield of 31.73% with inoculums load of 10^6 spores per ml.

Rhodesand Broderick (1989) carried out a 10 day fermentation of nitrogen wheat pollard and bran by a selection of filamentous fungi in stationary solid state culture at 63.5%

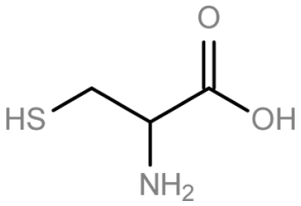
moisture and 30^{o}C and observed increases in crude protein percentage. Assessments of a range of concentrations of a variety of nitrogen supplements showed that ammonium phosphate (10% w/w) supported a maximal 170% increase in CP for a *Schizophyllum commune* fermentation. Poultry manure (1% w/w) supported a 155% in CP for *Aspergillus terreus* and a 126% increase for *Trichoderma reesi. Chaetomium virescens* concentration on unsupplemented wheat pollard and bran gave a 100% increase.

2.8.2 Effect of Sulphur supplementation on Protein Biomass Production

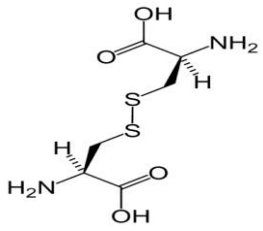
Sulphur (S) is an essential nutrient (micro-mineral) that is essential for the formation of many S containing compounds in the body. These compounds include the S containing amino acids (methionine, cysteine, cystine, homocysteine, cystathionine, taurine, cysteic acid), thiamin, biotin and lipoic acid (Bal and Ozturk, 2006).



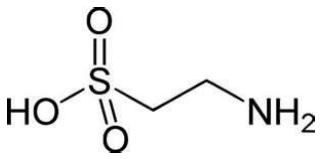
Methionine



Cysteine



Cystine



Taurine

Compounds containing sulphur play essential functions in the body as structural entities (collagen), catalysts (enzymes), oxygen carriers (hemoglobin), hormones (insulin), and as vitamins (thiamine and biotin). In most animals it represents about 0.25% of the body weight. Rumen microbial flora has an ability to convert inorganic S to organic S in the form of methionine, cysteine, and cystine. Therefore both organic and inorganic S sources are reduced to sulphide by rumen bacteria. The fate of the sulfide can be either microbial protein synthesis or absorption and oxidation to sulfate in liver (Bal and Ozturk, 2006).

Sulphur supplementation of sheep and cattle diets low in S has improved animal performance by increasing DM intake, weight gain, and S retention and wool growth (Fron *et al.,* 1990). Sulphate supplementation of untreated and alkali-treated wheat straws on ruminal fermentation in a semi continuous fermenter has been reported by Stevani *et al.* (1992).The S requirement of ruminants can be met by supplying either organic or inorganic S to ruminal bacteria because they convert inorganic S to organic compounds (Fron *et al.,* 1990).

Then S amino acids are synthesized from reduced form of S, called sulfide. Although it has been shown that some rumen bacteria need S amino acids for growth, sulfide has been shown to be a major source of S for bacterial protein synthesis. Gawthorne and Nader (1976) found in sheep that only 53 to 57% of the S in microbial protein came from the sulfide pool. They concluded that other half of the S amino acid content resulted from the direct incorporation of amino acids from digested plant and saliva.

The S requirement of lactating dairy cattle is 0.2% according to NRC (2001). In Australia, sulfur supplementation increased gain by 12% in steers grazing sorghum-Sudan grass containing 0.08 to 0.12 percent sulfur (Anonymous, 1998). The sulfur requirement of

ruminants grazing sorghum-Sudan grass may be increased because of the need for sulfur in the detoxification of cyanogen glucose found in most sorghum forages. Dietary sulphur requirements may be higher when diets high in rumen bypass protein are fed because of the limitation of sulphur for optimal ruminal fermentation. When urea or other non-protein nitrogen sources are fed, sulphur supplementation may be needed (Anonymous, 1998). Mature forage, forages grown in sulphur-deficient soils, corn silage, and sorghum-sudangrass can be low in suphfur. The typical nitrogen to sulfur ratio of a complete diet should be 10:1, nitrogen to sulphur (Anonymous, 1998).

2.8.2.1 Sources of Sulphur

Despite the fact that sulphur is a key mineral in many compounds essential for life, dietary inorganic sulfur is not necessary for the health of most animals. Pigs and poultry can do quite well with only organic sulfur (sulphur amino acids, thiamine, biotin, etc.) sources in their diets. The total absence of inorganic sulphur from the diet may increase the sulphur-amino acid requirement, which suggest that sulphur from the amino acids is used to synthesize other organic compounds containing sulphur.

Goodrich *et al.* (1978) reported that the nitrogen to sulphur ratio in rumen microbial protein averages 14.5:1. The common recommendation for the nitrogen: sulphur ratio is 10:1 in diets containing high levels of urea. The source of sulphur can influence its bioavailability. Sulphur supplements for ruminants (elemental, sodium sulfate and methionine) probably are utilized with different efficiencies and through alternate routes of metabolism. Goodrich *et al.* (1978) gave the following rankings from the most available to the least available: L-methionine > calcium sulfate > ammonium sulphate > sodium sulphate > molasses sulphur > sodium sulphide > lignin sulfonate > elemental sulphur. The recommended concentration of

sulphur in beef cattle diets is 0.15% (NRC, 1996). However, this assumes the sulphur source is highly bioavailable.

Sulphur supplementation from elemental S, sulphate and methionine resulted in similar increases in sulphur retention by heifers fed a tall fescue based diet, bioavailability was greater for sulphate and methionine than for elemental sulphur. Studies were carried out by Spear *et al*. (1978) to ascertain if supplemental sulphur (elemental or methionine sulphur) would influence ruminal microbial fermentation of Kentucky 31 tall fescue diets. The invitro study indicated that cellulose digestion of fescue by ruminant micro-organisms was stimulated by addition of either 0.05 or 0.15% sulfur to the substrate dry matter which contained 0.20% sulphur.

2.8.3 Effect of urea andmethionine supplementation on bacterial protein synthesis

Sulphate recycling becomes so critical when the exogenous S sources are not included in the diet in terms of fermentation and bacterial protein synthesis. So that when the rumen dietary N to rumen fermentable organic matter ratio declines in the case of low quality forage and starch, there is need to improve either sulphate recycling or exogenous S supplementation for protein synthesis. Therefore exogenous S sources become more critical for greater protein synthesis (Bal and Uzturk, 2006).

The role of S utilization (either organic or inorganic) in N-based diets has been considered for bacterial protein synthesis in terms of N: S ratio. It has been reported that a mean value of 18.5:1 N: S ratio for mixed rumen bacteria should be adequate to meet the S requirements of the rumen bacteria (Harrison and McAllan, 1980). However Harrison and McAllan

(1980) suggested that this N: S ratio may not be adequate to determine the requirement of bacteria due to the N and S escape. These losses was calculated using sheep, growing steers and lactating cows and concluded that these losses represent 85, 72 and 50% of the amount required for microbial protein synthesis, respectively (Kahlon *et al.*,1975). So that it is difficult to estimate how much ruminal sulphide is required for maximal microbial growth. It was also calculated that the requirements for available S in the rumen should be equivalent to 1.6-1.9 g/kg of digestible organic matter after S losses and recycling (Kahlon *et al*., 1975) It has been shown that an increase of S supply from 0.9 to 3.5 g/kg of rumen fermentable organic matter increased microbial N yield from 15 to 21 g/kg of rumen fermentable organic matter in semi continuous culture (Durand and Komisarczuk, 1988).

Kahlon *et al*. (1975) found that relative availabilities of calcium sulphate (94.1%) and ammonium sulfate (93%) had greatest sources for the in vitro bacterial protein synthesis (2.08 and 2.04 mg/ml of inoculum at 12 h, respectively). Sodium sulphate (55.4%) sodium sulphide (42.6%) and elemental S (35.8%) had lower capacity for in vitro protein synthesis (1.77, 1.69 and 1.62 mg/ml of inoculum at 12 h, respectively). Although there is a close relationship between N intake and the amount of protein produced by the rumen bacteria, it has been suggested that further protein synthesis can be improved by S supplementation. Another major aspect for influencing the utilization of ruminal S in microbial growth and protein synthesis is the availability of carbohydrate sources. As previously indicated, N: S ratio also becomes important in S supplemented diets for ruminal fiber degradation as well. Brondani *et al.* (1991) tested the additional effect of N and S together using high fiber diets for ruminal fermentation. They tried to achieve two different S level as sulfide (4 and 8 μg sulfide/ml of rumen fluid) using basal diet (0.2% S, DM basis) or supplemented with

elemental S at 0.2% of DM, and two different ammonia-N, supplemented as urea (5 and 15 mg ammonia-N/dl of rumen fluid) in the rumen. Brondani *et al.* (1991) set up four N: S treatment diets containing 3:1, 5:1, 8:1 and 12:1 using sugarcane bagasse and corn stover. They considered DMI and ruminal acetate as response elements and found that DMI did not differ among treatment groups. In both forage types, acetate production was not changed by either urea or S when fed separately. However when both urea and S were supplemented together, acetate production was 44% higher in sugarcane bagasse diet. There was 63% higher acetate production in corn stover diet. They concluded that S supplementation needs to be adjusted based on the N:S ratio and percentage of S in DM may not be adequate when high fiber diets containing low N were fed and suggested that high fiber diets low in N were utilized better when N and S were adequate.

Fazaeli *et al.* (2007), investigated the effects of three levels of urea and four levels of sulphur on in vitro digestibility and ferment ability of roughage based ration, incubated in rumen liquor of buffalo. They observed that invitro digestibility were not affected by the level of urea, sulphur or interaction between the two factors. There was no variation among the treatments up to 12h incubation for the cumulative gas production, but it was reduced when sulphur level increased from 0.37 to 0.41 or 0.45% of diet. They concluded that 0.33% sulphur and 0.5% urea which provided a total N/S ratio of 0.66 to 1.34 may be the optimum ranges for rumen fermentation of roughage based diets in buffalo.

Singh and Kishan (2003), reported that digestibility and nitrogen balance were significantly ($P<0.05$) higher in buffalo calves when compared with cattle calves. Niar *et al*. (2004) reported that bioavailability of CP and hemicellulose was improved in buffalo when fed roughage based diet supplemented with sodium sulphate. Sulphur supplementation of

roughages, which were mostly poor in available nitrogen and sulphur, was also essential to provide an optimum nitrogen sulphur balance for rumen microbial growth (Zinn *et al.*, 1997).

Fron *et al.* (1990) determine sulphur and nitrogen metabolism and bioavailability of different forms of supplemental S: elemental sulphur (E), sodium sulphate and DL-methionine (M) all at levels calculated to supply 0.15% S when growing Angus heifers were fed a ground fescue hay diet in a 72hr adaptation phase followed by a 5-day retention phase. They observed urinary S excretion during the 72hr period to be greater for S supplemented calves than for control. While no treatment differences ($P>0.01$) were noted in the VFA concentrations. S supplementation increased daily urinary sulphate ($P<0.10$), total urinary S ($P<0.01$) and fecal S ($P<0.01$) excretion during the retention phase. They concluded that supplementing S as elemental, sodium sulphate or methionine appeared to be equally beneficial, despite differences in route and extend of S excretion among the three sources.

2.9.0 The use of enzymes in monogastric nutrition

Enzymes are biological catalysts for organic reactions. They play key role in the digestive processes of the animal. They are proteins by nature and are produced by the animal itself or by the microbes that are naturally present in the digestive tracts. Their essential characteristic is to catalyze the rate of a reaction but is not themselves altered by it. They are involved in all anabolic and catabolic pathways of digestion and metabolism. Enzymes tend to be very specific catalysts that act on one or, at most, a limited group of compounds known as substrates. Enzymes are not living organisms and are not concerned about viability or cross infection (Khattak *et al.,* 2006*)*.

Enzymes play a key role in the digestive process. Although enzymes are produced by the animal itself or by the microbes naturally present in the digestive tract, specific activities necessary to break down some compounds in feed are not found or are at low levels in the digestive tract. Therefore, exogenous enzymes are added to the diet to break down these compounds. Feed enzymes are mainly produced from microorganisms by a process of fermentation and extraction. Throughout history, people have made use of enzyme-catalyzed reactions for such processes as brewing, baking, and antibiotic synthesis. Since the first enzyme - urease was isolated and purified by Sumner in 1926, isolated enzymes have been widely used in the textile, leather, food and beverage industries. Enzymes have been used in animal feed for more than 50 years, but the rapid growth in their use has only been within the last 10 years. The most common application of exogenous enzymes is in the feeding of the broiler chickens (Sheppy, 2001).

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.

2.9.1 Benefits of Enzymes supplementation in Poultry Rations

The benefits of using feed enzymes in poultry diets have been enumerated to include; reduction in digesta viscosity, enhancement of digestion and absorption of nutrients especially fat and protein, improvement in Apparent Metabolizable Energy (AME) value of the diet, increase in feed intake,

weight gain, and feed/gain ratio, reduction in beak impaction and vent plugging, decrease in the size of gastro-intestinal tract, alteration of population of microorganisms in gastro-intestinal tract, reduction in water intake and water content of excreta, reduction in the production of ammonia from excreta and reduction in the output of excreta, N and P (Marquardt *et al*. 1996; Ouhida *et al*, 2000; Gracia, *et al.,* 2003; Saleh, *et al.,* 2003 ; Odetallah, *et. al.,* 2005).

2.9.1.1 Reduction in Digesta Viscosity

Enzymes added to poultry diets; especially diets containing cereals rich in NSP such as wheat, barley, and rye, reduced viscosity in the diet and digesta. Morgan and Bedford (1995) and Muramatsu *et al*. (1992) found that enzyme supplementation of wheat based diets significantly reduced foregut digesta viscosity of birds. The reduction in foregut digesta viscosity was achieved primarily by reducing the molecular weight through hydrolysis of xylan backbone by endo-xylanase into smaller compounds and thus reduction in viscous effects of the feed because foregut digesta viscosity is directly proportional to the molecular weight of wheat arabinoxylans (Bedford and Classen, 1993). As a result of endo-xylanase and ß-glucanase supplementation, the long backbones of the arabinoxylans and ß-glucans were cleaved into shorter fragments, thereby reducing their viscosity (Gruppen *et al*. 1993). Similar findings on digesta viscosity were also reported by Bedford and Classen (1993); Bhatt *et al.* (1991) and Dunn, (1996) who inferred that the highviscosity in the gut contents caused by the pentosans ledto increased water intake of the birds, which resulted inthe wet and sticky droppings.

2.9.1.2 Increase in Available Energy

One of the main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization was associated with increased energy digestibility (Partridge and Wyatt, 1995). The AME of wheat has been extensively studied and found to have a considerable range from 9 500–16 640 kJ/kg (Mollah *et al*. 1983; Annison 1993; Choct *et al*. 1995). Enzyme supplementation improves this range by enhancing carbohydrate digestibility, reducing gut viscosity, and improving fat utilization (Almirall *et al*., 1995). The improvements in AME resulting from enzyme supplementation are variable because of the variability in the NSP content of wheat.

The increase in AME with the use of enzymes is difficult to predict, as nutrient ratios, such as energy–protein ratio, and other factors also play an important part in poultry-feed formulations. The AME value of wheat has been correlated with its content of water soluble NSPs (Annison and Choct, 1991), which in turn affects gut viscosity (Bedford *et al*., 1991). Unfortunately, NSP analyses are relatively lengthy processes, and in a commercial situation rapid testing of incoming grains is required. No chemical test or detectable physical characteristic can be used to rapidly predict the AME value of wheat or to estimate the improvements to be expected from the use of enzymes. This is part of the difficulty in trying to accurately estimate the energy content of wheat or barley in poultry feeds and compensate for the deficiency by adding enzymes. Adding adequate activity levels of *α*-amylase, *β*- glucanase, and xylanase to broiler starter and grower corn-soybean diets with a 3% reduction in dietary ME allowed full restoration of growth performance of broilers comparable to those fed the adequate energy (Yu and Chung, 2004).

2.9.1.3 Improvement in Nutrient Digestibility

Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley (Friesen *et al*., 1992; Marquardt *et al*., 1994), maize (Saleh *et al*., 2003), oats (Friesen *et al*., 1992), rye (Bedford and Classen 1992; Marquardt *et al*., 1994), and wheat (Marquardt *et al*., 1994), and to those containing pulses, such as lupins (Brenes *et al*., 1993). The effect of enzyme supplementation on Dry Matter Digestibility (DMD) in pigs and poultry depended on the type of diet and the type of animal: increases in DMD range from 0.9 to 17% (Annison and Choct, 1993) in poultry.

The enzymes currently used in monogastric diets are predominantly glycanases, which cleave NSPs into smaller polymers, thereby removing their ability to form viscous digesta and enhancing nutrient digestibility. The effects of glycanases are generally nonspecific, except for their effect on fat (greater effect on saturated fat than on unsaturated fat). Another enzyme used in feed is phytase, which increases the utilization of phytate phosphorus. The ability of phytase to improve the digestion of phytate phosphorus and subsequently to reduce the output of organic phosphorus to the environment has attracted a great deal of scientific and commercial interest. In poultry, use of phytase was reported to reduce phosphorus excretion by as much as 40% for broilers. When phytase was added to layer diets, increased egg production and positive effects on egg weight and tibia ash were also noted (Simons and Versteegh, 1991).

2.9.1.4 Reduction in Excreta Moisture

A reduction in the moisture content of poultry excreta is often noted when glycanases are included in the diet. Supplementing the NSP-enriched diet with three different commercial glycanase

products improved performance, but their effectiveness in reducing the moisture levels of the excreta differed from 10 to 29%. This supports the view that different glycanases have similar performance enhancement effects in monogastric animals but the site of the breakdown of the NSPs in the gut and the molecular sizes of the released products differed (Choct and Annison, 1990).

2.9.1.5 Health improvement

Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. Birds fed a wheat-based diet with and without glycanase supplementation showed vastly different responses to coccidiosis challenge. Growth was depressed by 52.5% in the control group but by only 30.5% in the enzyme group, which also had better lesion score. An increase in digesta passage rate and a reduction in excreta moisture were often noted when glycanases were added to poultry diets, which may be detrimental to the life cycle of the organism. Osman *et al*. (2007), mentioned that enzymatic growth promoters decreased wet litter syndrome, ammonia production in broiler houses and reduced the residence time of nutrients in the gastrointestinal tract by improving feed digestibility which gives less opportunity for growth of pathogenic bacteria.

2.9.1.6 Impact on environment

Enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore pose no threat to the animal or the consumer. Enzymes not only will enable livestock and poultry producers to economically use new feedstuffs, but will also prove to be environmentally friendly, as they reduce the pollution associated with animal production

(Hassanein, 2011). Enzyme inclusion also contribute to improvement in poultry production, and have a positive impact on the environment. In areas with intensive poultry production, the phosphorus output is often very high, resulting in environmental problems such as eutrophication. This happens because most of the phosphorus contained in typical feedstuffs exists as the plant storage form phytate, which is indigestible for poultry. The phytase enzyme frees the phosphorus in feedstuffs and also achieves the release of other minerals (e.g. Ca, Mg), as well as proteins and amino acids bound to phytate. Thus, by releasing bound phosphorus in feed ingredients, phytase reduces the quantity of inorganic phosphorus needed in diets, makes more phosphorus available for the bird, and decreases the amount excreted into the environment (Khattak *et al*., 2006).

2.9.2 Enzyme supplementation in poultry rations

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, *Apergillusniger* (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 *etal.*, 2003).

Many studies have been conducted to investigate the effect of carbohydrase and protease supplementation to corn-soy diets on the nutritive value of diets and performance of chicks. Marsman *et al.* (1997) examined the effect of enzyme treatments (protease and carbohydrase) of soybean meal on growth performance and ileal nutrient digestibilities in broiler chicks. Enzyme treatment improved apparent ileal digestibility of crude protein and NSPs; however, enzyme treatment did not improve growth performance of the chicks. Zanella *et al.* (1999) investigated the effect of a commercial enzyme cocktail containing xylanase, protease and amylase on performance of broilers fed a corn-soybean meal based diet. Enzyme supplementation improved body weight gain, feed conversion ratio and ileal digestibility of crude protein. Graham *et al.* (2002) pretreated soybean meal using 4% α-galactosidase enzyme solution. Enzyme treatment degraded raffinose and stachyose in soybean meal by 69 and 54%, respectively, compared to untreated soybean meal. Enzyme treatment increased TME from 2974 to 3328 kcal/kg. However, chick growth performance was not significantly improved by enzyme treatment. Kocher *et al.* (2003) investigated the effect of two enzyme products on the nutritive value of soybean meal with emphasis on changes in composition of NSPs along the digestive tract. They concluded that glycanases with galactanase and

pectinase activities supplemented at appropriate dosages can improve the digestibility of the NSPs in soybean meal and increase the metabolizable energy content of the diet containing high levels of soybean meal. In another report, Kocher *et al.* (2003) pointed out that although enzyme addition to the corn-soybean meal based diet can significantly improve AMEn, the improvement depended greatly on the raw ingredients available at the time. Studies by Ghazi *et al.* (2003) demonstrated the improvement of the nutritive value of soybean meal by protease and α-galactosidase treatment in broiler chicks. They first used tube-fed chicks to measure the effect of different enzyme treatments on true metabolizable energy (TME) and true nitrogen digestibility (TND) of commercial solvent-extracted, heat-treated soybean meal.

Protease and α-galactosidase improved TME and TND of the soybean meal. In other studies, they added enzymes in broiler diets and fed broilers for 21 d. Increases in chick growth rate and digestibility that were similar to those recorded in previous study were obtained when protease and α-galactosidase were included in the diets. Ritz *et al.* (1995) found a 3.6% improvement in body weight and 4.0% improvement in feed utilization in male turkeys when a corn-soybean meal diet was supplemented with α-amylase. The later study also demonstrated that the mean villus length within the jejunum and ileum was significantly increased at 2 and 3weeks of age by dietary supplementation of amylase when compared with control diet.

These findings suggest that the increased growth associated with the amylase diet be explained in part by the increase in absorptive surface area, allowing for increased digestion of available nutrients coupled with increased enzyme activity. However, no physiological mechanism to explain increased villus length as a response to enzyme supplementation is known. In chicks, Noy and Sklan

(1995) reported that daily net secretion of amylase was low at d 4 and steadily increased up to d 21. Gracia *et al.* (2003) studied the influence of exogenous α-amylase on digestion and performance of broilers fed a corn-soybean meal diet. At 7 d age, α-amylase supplementation improved daily gain by 9.4% and feed conversion by 4.2%. Also, α-amylase supplementation significantly improved apparent fecal digestibility of organic matter and starch and AMEn of the diet.

The weight of pancreas as a percentage of body weight decreased with α-amylase supplementation, which indicates that the secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine. Scheideler *et al.* (1999) conducted several studies to investigate the effect of enzyme addition to corn-soybean rations on pullet and laying hen performance. They used a commercial enzyme product called Avizyme 1500 that is a microbial multi-enzyme package with amylase, protease and xylanase activity. The results showed that the enzyme supplementation increased pullet growth rate and improved egg production, egg mass and feed conversion ratio. Improvements were also seen in nitrogen retention and availability of energy in pullet and layer diets supplemented with enzyme. In another study, Scheideler and Weber (2003a) investigated the role of α-galactosidase in corn-soy based layer rations. They found improvement in egg production of hens and the ME of the diet with the addition of α-galactosidase. Hens performed very well on reducing the energy available from fat sources and relying on more energy from soybean meal when α-galactosidase was added to the rations. Gomez *et al.* (1999) added Avizyme 1500 to corn-soybean meal diets with three energy densities. Enzyme supplementation improved egg mass and feed conversion ratio at all the three energy levels tested. Pretreatment of raw soybean or soybean meal with proteases was studied in many experiments. The purpose of adding proteases in soybean

or soybean meal containing diet is to destroy or inactivate the anti-nutritional factors, such as residual trypsin inhibitors, lectins and antigenic protein. Huo *et al.* (1993) found that fungal and bacterial protease enzymes could inactivate trypsin inhibitors and lectin in raw soybean and low-temperature extruded soybean *invitro*.

Based on their results, the protease from bacterial source was more effective at breaking down trypsin inhibitors than the protease from fungal source. Rooke *et al.* (1996) incubated the soybean meal using 0.1% acid protease for 3 h at 50°C, pH 4.5. The soybean meal treated with protease contained fewer antigenic proteins than the other soy-containing diets. In another study, they added alkaline protease into soybean meal and incubated for 2 h at 50°C, pH 8.5. They found the composition of soybean meal was changed due to pretreatment, and soluble α-amino nitrogen concentrations increased by treatment with protease and antigenic protein concentration was reduced. Beal *et al.* (1998a, b) reported that the pre-incubation of raw soybean or soybean meal with protease significantly increased the *in vitro* nitrogen digestibility.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 Experimental site and sources of experimental materials

The feeding trials were conducted at the Student Project Site of the Poultry Production Unit, College of Agriculture and Animal Science, Mando, Kaduna (11^0 10' N, $07^0$38'E with elevation of 632m above sea level. An average rainfall of 1200mm with 95% falling between April and October. The temperature varies between 26 -35 ^{o}c. The humidity at harmattan period is 21 and 27% at the wet season.). Mando is Located in the Northern Guinea savannah Zone of Nigeria(Googlemaps,2015)**.**

The sorghum beer residue was obtained from the local gin ("Burukutu") producers within Kaduna metropolis while fishmeal, groundnut cake and other feed ingredients were procured from Hassino Feed Millers Limited, Mando-Kaduna. Nigeria. Nutriad multi-enzyme was procured at Hybrid Feeds (Nig) Ltd., Television Village, Kaduna. While Zymos-N and Fullzyme multi-enzymes were procured at Animal Care Konsult, Kaduna

3.2.0 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 urea plus sulfur treated and fermented sorghum beer residue (RSBR) as well as microbial analysis of the wet RSBR samples. The proximate analysis was carried out at the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, according to AOAC (1990). The samples were analyzed for amino acid composition at the Department of Zoology, University of Jos, Nigeria. The microbial analysis was carried out at the Department of Microbiology, Ahmadu Bello University, Zaria.

The second phase of the experiment consisted of feeding trials. The growth performance and carcass characteristic of broiler chickens fed diets containing RSBR were evaluated in the first trial while in the second trial, growth performance and carcass characteristic of broiler chickens fed diets containing RSBRsupplemented with multi-enzymes were evaluated.

PHASE I

3.3.0 Sources and nature of Sorghum beer residue (SBR)

The SBR used in this experiment was the red sorghum spent grains. It is characteristically reddish in colour and appears fibrous. Dried samples are odorless with a sour taste. All samples used in the laboratory study and in experiments I and II, were purchased dried from the traditional 'Burukutu' brewers around Kaduna metropolis.

3.4.0 Determination of the effect on nutrient composition of sorghum beer residue passed through a 2nd fermentation process.

3.4.1 Inoculum preparation

Rumen content of cow was collected fresh just after slaughter at the Sabon-Tasha abattoir, Kaduna. This was then mixed with water in a 2:1 ratio on a weight by weight (w/w) basis and filtered through a mosquito net of pore size 2.78mm^2 (Ndams *et al.,* 2011). The residue was discarded and the filtrate used as inoculum. The method of inoculum preparation for SBR inoculation and fermentation in experiments I and II were similar to what was done here.

3.4.2 Substrate inoculation

Four (4) different concentrations of substrate to inoculum ratios were used (10:1, 10:2, 10:3, and 10:4 w/w). The 4different inoculated samples were each subjected to 4 days fermentation. Four bottles (empty jam containers with covers) of equal volume were used. Each SBR inoculated sample (10:1, 10:2, 10:3, and 10:4)was subjected to 4 days fermentation (Ndams *et al*, 2011). The four (4)bottles were used to carry the same quantity of inoculated SBR sample. The quantity of water that was used according to Ndams *et al*. (2009) to wet a given quantity of dried SBR was in a ratio of 5:1(w/w). The 4different inoculum concentrations (10:1, 10:2, 10:3, and 10:4) were first introduced into the water before wetting the SBR samples for uniformity of inoculation (Ndams *et al*., 2011).

Quantitatively, 4buckets were kept and 500g of SBR were weighed into them. The following weights of inoculum (rumen liquor); 50, 100, 150, and 200g corresponding to the substrate to

inoculum ratio of 10:1, 10:2. 10:3 and 10:4 were weighed and introduced into equal volume of water (2500g). This was properly mixed and each volume of water containing the different concentration of inoculum was used to wet the 500g of SBR in the bucket in a ratio of 1:5(w/w). One bottle was allocated to each of the four different SBR inoculated sample to which 500g of the SBR inoculated sample was filled. The samples were then kept under room temperature to ferment for a period of four days. The different ratios used were as presented in Table 3.1

Table 3.1: The ratio of substrate to water and to inoculum concentration

Sample	Substrate to water ratio	Substrate to Inoculum ratio
A	1: 5 (500g: 2500g)	10: 1 (500g: 50g)
B	1: 5 ” ”	10: 2 (500g: 100g)
C	1: 5 ” ”	10: 3 (500g: 150g)
D	1: 5 ” ”	10: 4 (500g: 200g)

3.4.3 Fermentation

Each inoculated SBR sample was properly compacted into the bottle to exclude any possible air. The mouth of the bottles were covered with polythene materials and finally sealed with covers so as to maintain anaerobic condition as much as possible. All bottles of the inoculated SBR samples were labeled accordingly and allowed to ferment for 4 days.

After the period of fermentation (4days), 10 g of the re-fermented SBR (RSBR) sample was taken into text tubes. The four (4) test tubes containing wet re-fermented SBR material were properly labeled and kept in a refrigerator for bacteria count. The rest of the re-fermented SBR 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 Department of Animal Science, A.B.U. Zaria.

3.5.0 Proximate analysis

Proximate analyses were carried out on the SBR sample and the 4 RSBR samples for dry matter (DM), ash, ether extract (EE), crude fibre (CF), nitrogen (N_2) and crude protein (CP) determination according to the method of AOAC (1990). NFE was calculated by difference.

3.6.0 Microbiological Analysis

Microbiological analysis was carried out at the Department of Microbiology, A.B.U Zaria to identify the substrate to inoculum concentration that will result in the highest bacteria count. The procedure was as follows:

3.6.1 Media preparation

Nutrient agar (bacteria growth media) (12.4g) was added to 450 ml of distilled water and mixed. The media contained in a beaker was 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 pouring into plates.

5.6.2 Preparation of the dilution

Each wet RSBR sample (1.0g) was weighed and mixed in 100ml sterile distilled water and out of this, 10 ml, was taken in a test tube as the stock bacteria concentration. This contained 0.lg of the RSBR sample (0.1g/10ml).

3.6.3 Serial dilution

To reduce the bacteria concentration before inoculation of every sample, five (5) 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 5. From the stock concentration, 0.l ml was removed and transferred into the first dilution blank as shown in Figure 3.1. These were mixed and from it 0.1ml was removed and discarded every time to allow equal concentration.

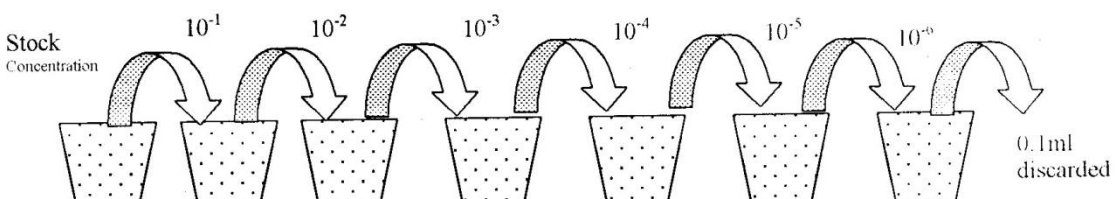


Fig 3.1. Serial dilution of bacteria stock solution

3.6.4 Inoculation of plates

The 5th dilution was used for plate inoculation. In the process, 1ml of the 5^{th} dilution was removed with a pipette and introduced into the center of each sterile petri-dish. 10ml of nutrient agar (cooled to about 45^0C) was poured on each plate and mixed by moving the dish gently, six times in clockwise direction and repeated counter clock wisely. After, the media was allowed to set for 20 minutes and the plastic dishes were inverted and incubated at 37 ^{0}C for 24hrs.

3.6.5 Bacteria population count on plates

The plates were divided into four quarters as shown in Figure 3.2. Each quarter was counted and the count of the four quarters added to obtain the total count (Count per plate = a + b + c + d).

a *b*

c d

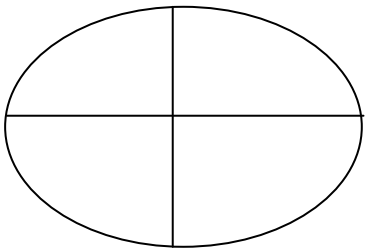


Fig.3.2 : Bacteria growth plate divided into four quarters

3.6.6 Chemical analysis

The rest of the re-fermented SBR (4 samples) were removed from the bottles and sun-dried for 3 days after which samples were taken for chemical analysis at theDepartment of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. The proximate determination for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash and nitrogen free extract (NFE) were carried out. The analytical procedures for DM, CF, CP, EE, Ash and NFE determinations employed in this experiment were in accordance with AOAC (2000). The amino acid profile of the sample with substrate to inoculums concentration that yielded the highest crude protein content were determined at the Department of Zoology, University of Jos using methods described by Sparkman *et al*.(1958).

The substrate to inoculums concentration of the sample that yielded the highest crude protein content was noted for application in experiments 2, 3 and 4. This was done in ratio 10:1 substrate to inoculum concentration.

3.7.0 Determination of the effect on nutrient composition of SBR supplemented with urea and passed through a 2nd stage fermentation process.

Four (4) SBR samples of same substrate to inoculums concentration (10:1) were supplemented with the following levels of urea nitrogen on dry matter basis; 0.5, 1.0, 1.5, and 2.0% (Fazaeli

et al, 2007; Isekwenu *et al*, 2007) before re-fermentation for 4days. The quantity of urea required by weight at different levels for the treatment of 500 grams SBR samples were as follows; 2.5miligrams (mg), 5.0mg, 7.5mg and 10.0mg. The different ratios used are as presented in Table 3.2

Table 3.2: Ratio of urea to water to inoculum and to substrate

Sample	Urea (mg)	Water (g)	Inoculum (g)	SBR (g)
A	2.5	2500	50	500
B	5.0	2500	50	500
C	7.5	2500	50	500
D	10.0	2500	50	500

Urea samples were always dissolved in water and inoculum added before the water was used in wetting the SBR sample to allow uniformity. The samples were packed in jam bottles and allowed to ferment for four days.

3.8.0 Determination of the effect on nutrient composition of SBR supplemented with methionine and passed through a 2nd fermentation process.

Four (4) SBR samples of same substrate to inoculums concentration (10:1) were treated with the following levels of methionine on dry matter basis; 0.29, 0.33, 0.37, and 0.41% (Fazaeli *et al.,* 2007)

before re-fermentation for four days. The levels of methionine required for the treatment of 500g of SBR were as follows: 1.45, 1.65, 1.85 and 2.00mg. The different ratios used were as presented in Table 3.3

Table 3.3: Ratio of methionine to water, to inoculum, and to substrate

Sample	methionine (mg)	Water (g)	Inoculum (g)	SBR (g)
A	1.45	2500	50	500
B	1.65	2500	50	500
C	1.85	2500	50	500
D	2.00	2500	50	500

Methionine was first dissolved in the water and the inoculum introduced before the SBR samples were wetted. The 500g SBR sulphur treated samples were compacted in jam bottles and fermented for 4 days.

3.9.0 Determination of the effect on nutrient composition of SBR supplemented with urea and methionine and passed through a 2nd stage fermentation process.

Sixteen (16) SBR samples of same inoculum concentration (10:1) were treated with different levels of urea and methionine on dry matter basis and then subjected to 4 days fermentation. The levels of urea and methionine used are as presented in Table 3.4

Table 3.4: Levels of urea and methionine treatments on sorghum beer residue with same substrate to inoculums concentration

	*Levels of urea (%)			
Levels of Methionine (%)	**N_1**	**N_2**	**N_3**	**N_4**
S1	0.29(0.5)	0.29(1.0)	0.29(1.5)	0.29(2.0)
S2	0.33(0.5)	0.33 (1.0)	0.33(1.5)	0.33(2.0)
S3	0.37(0.5)	0.37 (1.0)	0.37(1.5)	0.37(2.0)
S4	0.41(0.5)	0.41 (1.0)	0.41(1.5)	0.41(2.0)

*Values in parentheses are amount of urea (on percentage dry matter basis)

Quantitatively, the different levels of urea and methionine required to treat 16 SBR samples (500g each) are as shown in table 3.5

Table 3.5: Quantities (g) of urea and methionine treated with Sorghum Beer Residue samples

	*Quantity of urea (g)			
Quantity of methionine (g)	**N_1**	**N_2**	**N_3**	**N_4**
S_1	1.45(2.5)	1.45(5.0)	1.45 (7.5)	1.45(10.0)
S_2	1.65(2.5)	1.65(5.0)	1.65(7.5)	1.65(10.0)
S_3	1.85(2.5)	1.85(5.0)	1.85(7.5)	1.85(10.0)
S_4	2.00(2.5)	2.00(5.0)	2.00(7.5)	2.00(10.0)

*Values in parentheses are amount of urea (on percentage dry matter basis)

Chemical analysis and bacteria count were carried out on all samples for experiment ii, iii and iv. The best sample among the sixteen (16) samples supplemented with urea (N) and methionine (S) that yield the highest crude protein content was selected for the determination of amino acid profile. The urea/methionine ratio that yielded the highest crude protein was marked as the optimum urea/methionine (N/S) ratio for SBR fermentation. The N/S ratio was used in SBR supplementation in carrying out bulk fermentation for on-farm feeding trials.

3.10.0 Processing of sorghum beer residue for On-farm feeding trials

The inoculation of sorghum beer residue prior to fermentation was carried out as described by Ndams *et al*. (2011). SBR was moistened with water at a ratio of 1:5 (w/w) SBR was supplemented

with urea and methionineconcentrations at a ratio of 1.5: 0.37 % (established from the preliminary study). The quantities of urea and methionine were first dissolved in the water containing the inoculum before wetting the substrate (SBR).

On quantitative measurement, 2.67kg of methionine and 10.80kg of urea were dissolved in 3600kg of water in which 72.00kg of rumen liquor has been introduced before wetting 720kg (twelve bags of 60 kg each) of SBR. The SBR was inoculated on concrete floor and then turned into a 5ft deep pit lined with polythene materials. It was properly compacted to maintain perfect anaerobic condition. The urea and methionine supplemented SBR was allowed to ferment for 4days. After fermentation, the pit was opened, and observations were made on the contents. The fermented product (RSBR) was sundried for 7days on concrete floor and samples taken for proximate analysis according to AOAC (2000) procedures for DM, CP, CF, EE, Ash and NFE at the Department of Animal Science, A.B.U. Zaria.

3.10.1 Determination of amino acid composition

The amino acid profiles of the SBR sample, the highest protein biomass produced from fermented urea, methionine and urea plus methionine supplemented samples were determined using methods described by Sparkman *et al*. (1958).

PHASE II

FEEDING TRIALS

3.11.0 Experiment 1. Effect of feeding urea and methionine-treated and fermented sorghum beer residue on the performance and carcass characteristics of broiler chickens

The performance of broilers was evaluated in an 8-week feeding trial where fermented nitrogen plus sulfur supplemented SBR (as a test ingredient) was used to replace groundnut cake (GNC) at 0, 25, 50, 75 and 100% levels on protein equivalent basis during the starter and finisher phases. The diets were least-cost formulated in order to meet the recommended nutrients requirements for starter and finisher broiler birds by NRC (2000). The ingredient composition of the broiler starter and finisher diets are as shown in Tables 3.6 and 3.7respectively.

3.11.1 Broiler starter phase (0- 4 weeks)

Three hundred and forty five (345) day-old broiler chicks were used. They were obtained from Yammfy Farms (Nig) Ltd. Ilorin, Nigeria. The birds were randomly allocated to 15 pens in groups of 23 birds per pen. Five diets were formulated in which RSBR was fed at GNC replacementlevels of 0, 25, 50, 75 and 100%. Each dietary group was replicated three times. The diets were randomly allocated to the pens of broilers on concrete flooring; each pen measuring 3.5 by 7.0m. Wood shaving was used as bedding materials.

All the test rations were iso-nitrogenous and iso-caloric. The starter diets were formulated to have 23% CP. The respective groups of chickens were fed the starter diets (Table 3.6)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

Table 3.6: Ingredient composition (%) of broiler starter diets containing replacement levels of urea plus methionine supplemented and fermented sorghum beer residue(0-4 weeks)

	Levels of RSBR inclusion (%)				
Ingredient (%)	**0**	**25**	**50**	**75**	**100**
Maize	43.20	40.98	39.99	33.10	30.05
Maize offal	10.00	10.00	10.00	10.00	10.00
RSBR	0.00	9.60	21.35	35.26	46.57
GNC	37.55	28.97	18.20	9.65	0.00
Fishmeal	3.50	3.50	3.50	3.50	3.50
Bone meal	2.50	2.50	2.50	2.50	2.50

Limestone	0.50	0.50	0.50	0.50	0.50
Palm oil	1.50	2.50	2.68	4.20	5.55
Lysine	0.40	0.42	0.43	0.44	0.48
Methionine	0.25	0.25	0.25	0.25	0.25
[1]Vit/Min Premix	0.30	0.30	0.30	0.30	0.30
Common Salt	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
ME. Kcal/kg 2872.31		2867.37	2854.13	2845.35	2844,52
CP (%)	23.07	22.78	22.56	22.91	22.56
EE (%)	5.77	5.04	4.19	3.57	2.59
CF (%)	4.31	5.38	5.99	6.74	7.71
Ca (%)	1.32	1.33	1.34	1.36	1.37
Avail. P	0.89	0.89	0.88	0.88	0.87
Lysine (%)	1.28	1.25	1.25	1.25	1.25
Met.+Cys.(%)	0.87	0.96	1.06	1.16	1.26
Cost ₦/kg	104.06	99.72	93.26	90.32	84.57

[1]Vitamin/ mineral premix (Animal Care.Optimix[R]) Each 1.25kg supplied: Vit A 12,000,000 I.U., D_3 3000,000 I.U; Vit. K2500mg; B_1, 200mg; B_2 500mg; B_6 3,500mg; Niacin 40,00mg; B_{12} 20mg; Pantothenic acid 10mg; Folic acid 1,000mg; Biotin 80mg; Choline chloride 200,000gm; Anti-oxidant 125,000mg; Manganese 70,000gm; Iron 40,000gm; Copper 8000mg; Iodine 1,200mg; Selenium 250mg; Cobalt 250mg.

RSBR = Refermented sorghum beer residue, GNC = Groundnut cake

commencement of the experiment and subsequently at weekly intervals. The record of feed

consumption was also kept. Natural day light was used immediately after brooding. Record of mortality was kept as it occurred.

3.11.2 Broiler finisher phase (4-8 weeks)

At the end of the starter phase, the different groups (20 birds each) were carried through to the finishing stage. The same number of pens (15) 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, 25, 50, 75 and 100% RSBR respectively (Table 3.7). Feed and water were provided *ad libitum.* Weekly records of live weight were kept from which the body weight gain per day (growth rate) was determined. Feed intake, feed to gain ratio and feed cost per kilogram gain were calculated. Mortality was recorded as it occurred. The finisher phase lasted for 4weeks.

Table 3.7: Ingredient composition (%) of broiler finisher diets containing replacement

levels of urea plus methionine supplemented and fermented sorghum beer residue(4-8 weeks)

	Levels of RSBR inclusion (%)				
Ingredient (%)	**0**	**25**	**50**	**75**	**100**
Maize	48.00	43.85	42.50	42.40	40.11
Maize offal	10.00	10.00	10.00	10.00	10.00
RSBR	0.00	7.90	18.05	24.30	36.39
GNC	32.40	25.90	17.00	10.85	0.00
Fishmeal	3.80	3.80	3.80	3.80	3.80
Bone meal	2.50	2.50	2.50	2.50	2.50
Limestone	0.40	0.40	0.40	0.40	0.40
Palm oil	1.82	4.50	4.50	4.50	5.55
Lysine	0.40	0.40	0.40	0.40	0.40
Methionine	0.25	0.25	0.25	0.25	0.25
[1]Vit/Min Premix	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
ME.kcal/kg	2948.09	3027.18	2963.91	2947.09	2945.31
CP (%)	20.53	20.52	20.47	20.46	20.50
EE (%)	5.49	4.86	4.13	3.66	2.76
CF (%)	4.32	5.21	5.61	6.16	7.12
Ca (%)	1.28	1.29	1.30	1.31	1.32
Avail.P(%)	0.88	0.87	0.87	0.86	0.86
Lysine(%)	1.23	1.18	1.17	1.15	1.20
Met. + Cyst. (%)	0.83	0.91	1.01	1.05	1.16
Cost ₦/kg	103.62	103.61	96.91	92.72	86.89

[1]Vitamin/ mineral premix (Animal Care.Optimix[R]) Each 1.25kg supplied: Vit A 12,000,000 I.U; D_3 3000,000 I.U;Vit. K2500mg;B_1, 200mg; B_2 500mg; B_6 3,500mg; Niacin 40,00mg; B_{12} 20mg; Pantothenic acid 10mg; Folic acid 1,000mg; Biotin 80mg; Choline chloride 200,000gm; Anti oxidant 125,000mg; Manganese 70,000gm; Iron 40,000gm; Copper 8000mg; Iodine 1,200mg; Selenium 250mg; Cobalt 250mg.

RSBR = Refermented sorghum beer residue, GNC = Groundnut cake

3.11.3 Carcass and haematological characteristics of broilers fed RSBR (0-8weeks)

At the end of the broiler finisher phase (8-weeks), three birds from each pen whose weights were equal or close to the mean weight of the birds in the pen were selected. The sampled birds were sacrifice for carcass analysis. The selected birds were slaughtered by severing the neck with a sharp knife and allowed to bleed. 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. Similarly, from the birds selected to be sacrificed for carcass analysis, one bird was randomly selected from each replicate, making a total of 15 birds for the collection of blood samples for haematological assay. 2mls of blood from each bird was collected into bottle treated with ethylene diamine tetra acetic acid (EDTA). The bottles were labelled accordingly and taken for haematological evaluation at the Veterinary Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Blood samples were analyzed within 6 hours of collection for haematocrit or parked Cell volume (PCV), haemoglobin (Hb) according to the methods described by Dein (1984).

Parked cell volume (PCV) was measured as micro haematocrit with 75 x 16mm capillary tubes filled to 2/ng level with blood and centrifuged at 3000 rpm for 5 minutes. Haemoglobin concentration (g/dl) was also calculated. The total serum protein (TP) was determined by the Goldberger refractometer to obtain concentration (g/dl) per blood sample.

3.11.4 Data analysis

Data collected were subjected to Analysis of variance procedure. (ANOVA-Single factor) using "Analyse-it'' for Microsoft Excel (3.03) Standard. Standard. Significant means were separated using the Duncan Multiple Range Test (1995) taking $P < 0.05$ as significance level

3.11.5 Cost Analysis

Calculations of the cost of all ingredients used in this study were carried out based on current market prices as at the month of May, 2015. The emphasis however, was placed on the economic analysis to demonstrate the cost benefit of using the new feedstuff (RSBR). Price of RSBR was established by taking into consideration of the various costs associated with obtaining the waste residue. These included labor cost for collection, transportation and processing.

3.12.0 The performance of broiler chickens fed 75% urea and methionine treated and refermented sorghum beer residue diets supplemented with specific multi-enzymes

Having observed from experiment 1 the depressed live weights and feed efficiency of broilers fed 75 and 100% RSBR diets, in experiment II the performance of broilers was evaluated in an 8-week feeding trial where 75% urea and methionine treated and refermented sorghum beer residue diets supplemented with specific multi-enzymes was fed. The diets were least-cost formulated in order to meet the recommended nutrients requirements for starter and finisher broiler birds by NRC (2000).

3.12.1 Experimental design and dietary treatments

The experimental period was divided into two feeding phases, starter phase (from 0-4weeks of age) and finisher phase (from 5-8 weeks of age). All diets were formulated to meet requirements of broiler chickens according to NRC (1994). The enzymes used in this experiment were; Zymos-N® (Vamso, Biotec Pvt. Ltd Chennai, India) and Fullzyme® (Biofeed Technology INC Quebec, J4Y 2R3 Canada) and Nutriad® (efeedBiz Co. Hoogveld 93, Dendermonde, Belgium).

Nutriad® enzyme is a multi-enzyme compound of □-glucanase, xylanase, hemicellulase, cellulase, pectinase, proteinase, anti caking agent, sepittite (E562), mineral substance and activated yeast extract. Zymos-N is a multi-enzyme compound of □-glucanase, xylanase, amylase, cellulase, Proteinase, lipase, phytase, pectinase, , lipase, ɑ- galactosidase, □-galactosidase, mannoglucans and yeast nucleotide.

Fullzyme® is a multi- enzyme compound of fungal protease, ɑ-amylase, cellulase, lipase,

?-galactosidase, phytase, pectinase, xylanase, Bacillus substilis fermentation extract and Aspergillus orizae.Nutriad®, Zymos-N® and Fullzyme® multi-enzymes were supplemented at the level of 500g/ton, 300g/ton and 250g/ton of feed respectively as recommended by the manufacturers.

3.12.2The starter phase (0-4 weeks)

Three hundred andforty five (345) day old-broiler chicks of mixed sexes were used for this experiment. The chicks were weighed at day-old and randomly allocated to 15 pens in groups of 23 birds per pen. Five 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 (0-4) weeks. The chicks were brooded conventionally in a deep-litter house. Electric bulbs (200 watts) and charcoal pots were used as sources of heat. The starter diets were iso-nitrogenous (23.0% CP) and iso-caloric (3000.00 kcal/kg). The diets for the starter phase as shown in Tables 3.8.

Diet 1 was the control diet. It had no 75% RSBR and no enzyme. Diet 2 contained 75% RSBR but without enzymes supplementation. Diet 3 contained 75% RSBR plus 0.05% Nutriad enzyme supplementation. Diet 4 contained 75% RSBR plus 0.03% Zymos-N enzyme supplementation.Diet 5contained 75% RSBR plus 0.03% Fullzyme enzyme supplementation. The treatments were arranged in a completely randomized design. 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 intervals. The

record of feed consumption was also kept. Feed conversion (gram feed/gram gain) was calculated for the experimental period. Mortality was recorded as it occurred and the percentage calculated per treatment group for the entire experimental period (4-weeks). Live weight, average daily gain, feed intake, and feed cost per kilogram gain ~~(N)~~ were calculated for the period.

Table 3.8: Ingredient composition (%) of 75% urea and methionine treated and fermented sorghum beer residuediets supplemented with multi-enzymes (Starter phase)

			Treatments		
			75%RSBR	75%RSBR	75%RSBR
Ingredients	**0% RSBR**	**75% RSBR**	**+Nutriad**	**+ Zymos-N**	**+Fullzyme**
Maize	39.00	33.20	33.15	33.17	33.17

Maize offal	10.00	9.00	9.00	9.00	9.00
RSBR	0.00	34.90	34.90	34.90	34.90
GNC	38.25	7.00	7.00	7.00	7.00
SBC	6.00	6.00	6.00	6.00	6.00
Bone meal	2.50	2.40	2.40	2.40	2.40
Limestone	0.70	0.70	0.70	0.70	0.70
Palm oil	2.30	5.50	5.50	5.50	5.50
Lysine	0.40	0.45	0.45	0.45	0.45
Methionine	0.25	0.25	0.25	0.25	0.25
Vit/Min Premix[1]	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Nutriad	0.00	0.00	0.05	0.00	0.00
Zymos-N	0.00	0.00	0.00	0.03	0.00
Fullzyme	0.00	0.00	0.00	0.00	0.03
Total	**100.00**	**100.00**	**100.05**	**100.03**	**100.03**

Calculated Analysis

Crude protein,(%)	23.10	22.81	22.81	22.81	22.81
ME.Kcal/kg	2948.09	2850.25	2848.02	2848.02	2848.02
Ether extract, (%)	5.66	3.02	3.02	3.02	3.02
Crude fibre, (%)	4.43	7.13	7.13	7.13	7.13
Calcium, (%)	1.26	1.30	1.30	1.30	1.30
Avail. P, (%)	0.82	0.79	0.79	0.79	0.79
Lysine, %	1.31	1.25	1.25	1.25	1.25
Met. + Cys, (%)	.83	0.86	1.16	1.16	1.16

Cost ₦/kg	92.79	74.61	75.61	76.99	76.56

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 4g;Niacin 40mgm; B_{12} 0.02mg; Pantothenic acid 10mg; Folic acid 1mg; Biotin 88mg; 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; Cobalt 0.240mg.

RSBR = Refermented sorghum beer residue. GNC = Groundnut cake, SBC = Soya bean cake

3.12.4 Finisher Phase (4-8 weeks)

At the end of the starter phase (4-weeks of age), the birds were pooled together and three hundred (300) birds were randomly re-assigned to the experimental pens, balancing for mean weights in each of the 15 experimental pens. There were also five 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 twenty (20) birds per pen. The finisher test rations were formulated to be iso-caloric and iso-nitrogenous at 20.5% CP. The composition of the finisher diets is as shown in Table 3.9.

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 were kept and calculated. The finisher phase lasted from 4to 8 weeks.

Table 3.9: Ingredient composition (%) of 75% refermented sorghum beer residuediets supplemented with multi-enzymes (Finisher phase)

	Treatments				
Ingredients	**0% RSBR**	**75% RSBR**	**75%RSBR +Nutriad**	**75%RSBR + Zymos-N**	**75%RSBR +Fullzyme**
Maize	45.05	40.20	40.15	40.17	40.17

Maize offal	10.00	10.00	10.00	10.00	10.00
RSBR	0.00	24.25	24.25	24.25	24.25
GNC	31.00	9.60	9.60	9.60	9.60
SBC	6.00	6.00	6.00	6.00	6.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Limestone	0.70	0.70	0.70	0.70	0.70
Palm oil	3.50	5.5	5.5	5.5	5.5
Lysine	0.40	0.45	0.45	0.45	0.45
Methionine	0.25	0.25	0.25	0.25	0.25
Vit/Min Premix[1]	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Nutriad	0.00	0.00	0.05	0.00	0.00
Zymos-N	0.00	0.00	0.00	0.03	0.00
Fullzyme	0.00	0.00	0.00	0.00	0.03
Total	100.00	100.00	100.05	100.03	100.03

Calculated Analysis

Crude protein,(%)	20.53	20.54	20.54	20.54	20.54
ME.Kcal/kg	2948.09	2952.82	2952.82	2952.82	2952.82
Ether extract, (%)	5.49	3.69	3.69	3.69	3.69
Crude fibre, (%)	4.32	6.18	6.18	6.18	6.18
Calcium, (%)	1.28	1.30	1.30	1.30	1.30
Avail. P, (%)	0.88	0.86	0.86	0.86	0.86
Lysine, %	1.23	1.19	1.19	1.19	1.19
Met. + Cys, (%)	.83	1.05	1.05	1.05	1.05

Feed cost ₦/kg	87.12	75.74	76.51	78.12	77.69

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 88mg; 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; Cobalt 0.240mg.

RSBR = Refermented sorghum beer residue. GNC = Groundnut cake, SBC = Soya bean cake

3.12.5 Carcass evaluation of finished broilers

At the end of the eight-weeks feeding trial (at 56 days of age), birds were fasted overnight with access to water. Three birds from each pen with approximate body weight equal to the mean weight of the birds in the pen were randomly 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, de-feathered and eviscerated. After removal of the head, neck, viscera, shanks, spleen, digestive tract, liver, heart, gizzard and abdominal fat, the rest of the body was weighed to determine the dressed weight. Dressing percentage was calculated as (dressed weight/live weight) x 100). The weight of the breast, thigh, back, wing, liver, heart, empty gizzard, pancreas and abdominal fat were recorded. These were expressed as percentages of live weight. The intestinal length was measured in centimeters.

3.12.6 Statistical analyses

Data collected were subjected to Analysis of variance procedure. (ANOVA-Single factor) using "Analyse-it'' for Microsoft Excel (3.03) Standard. Significant means were separated using the Duncan Multiple Range Test (1995) taking P <0.05 as significancelevel.

3.12.7 Cost Analysis

Calculations of the cost of all ingredients used in this study were carried out based on current market prices as at the month of August, 2015. The emphasis however, was placed on the economic analysis to demonstrate the cost benefit of using the new feedstuff (RSBR). Price of RSBR was established by taking into consideration of the various costs associated with obtaining the waste residue. These included labor cost for collection, transportation and processing.

CHAPTER FOUR

4.0 RESULTS

4.1.1 Laboratory Studies

4.1.2 The effect of 2nd stage fermentation process on nutrient composition of SBR

4.1.2.1 Physical characteristics of urea and methionine supplemented refermented SBR (RSBR)

The dried sorghum beer residue (SBR) procured was fibrous, brownish in colour and barely odorless. But on opening the pit at the end of the 4days fermentation, the fermented material (RSBR) was hot when touched. The texture became smoother and colour change was observed from brown to dark brown. Before drying, it had a sweaty odour comparable to that of a ripened corn silage.

4.1.3 Effect of different inoculum concentration on nutrient composition of SBR after 4 days fermentation

Table 4.1 shows the effect of SBR with different levels of inoculum concentrations on the proximate composition of SBR. In terms of dry matter (DM) composition, sample with substrate to inoculum concentration of 10:1 was significantly higher ($P<0.05$) compared to those on 10:2, 10:3 and 10:4, but significantly lower ($P<0.05$) to the control. SBR samples on 10:2 and 10:3 concentrations were similar but significantly higher ($P<0.05$) than those on 10:4 substrate to inoculum concentration. The Crude Protein (CP) content of fermented sample on 10:4 substrate to inoculum concentration was significantly higher ($P<0.05$) than the control and the fermented samples on 10:2 and 10:3 but similar to 10:1 substrate to inoculum concentrations. The CP content of purchased SBR sample (control)and 10:1 concentration were similar but

Table 4.1 Effect of different inoculum concentration on sorghum beer residue proximate composition (%) after 4day fermentation

	Substrate to inoculum concentration					
Parameters (%)	**0[1]**	**10:1**	**10:2**	**10:3**	**10:4**	**SEM[†]**
Dry matter,	91.76[a]	91.16[b]	89.78[d]	89.49[d]	90.41[c]	0.42
Crude protein,	21.73[b]	22.10[ab]	20.72[c]	19.99[d]	22.41[a]	0.45
Ether extract,	1.20[c]	2.78[a]	2.54[a]	2.80[a]	2.22[b]	1.03
Crude fibre,	14.49[a]	10.23[b]	8.07[c]	10.82[b]	10.95[b]	0.30
ASH,	24.36[a]	16.94[b]	6.18[c]	6.71[c]	6.53[c]	3.6
NFE	37.43[c]	39.54[b]	39.29[b]	34.69[d]	42.21[d]	1.25

[a, b, c, d] Means of the same row with different superscripts are significantly different (P<0.05)
*Values represents averages of three determinations per sample
[1]Unfermented sorghum beer residue (control)
[†]Standard error of mean

significantly higher (P<0.05) than those on 10:2 and 10:3 substrate to inoculum concentrations. The CP percentage of Sample on 10:3 was significantly (P<0.05)lower thansample on 10:2 substrate to inoculum concentration. The percentage ether extract of fermented SBR samples on 10:1, 10:2 and 10:3 were similar (P>0.05) but significantly (P<0.05)higher compared to those on control and 10:4 substrate to inoculum concentration. The crude fibre percentage (CF,%) of fermented SBR samples on 10:1,10:3 and 10:4 concentrations were similar (P>0.05) but significantly (P<0.05)lower than the control. Sample on 10:2 had the least CF. The ash content of fermented SBR samples on 10:2, 10:3 and 10:4 were similar (P>0.05) but significantly (P<0.05)lower than those on 10:1 and the control. The control was significantly (P<0.05) higher than sample on 10:1

4.1.5 Effect on nutrient composition of SBR with urea supplementation after 4 days fermentation

Table 4.2 shows the effect on nutrient composition of urea treated and fermented SBR. The DM composition of samples treated with 0.5% urea was significantly (P<0.05) higher compared to those on 1.0, 1.5, and 2.0% urea treatment but significantly (P<0.05) lower to the control. The DM composition for samples on 1.5 and 2.0% were similar (P>0.05) but significantly (P<0.05) lower than that of 1.0% urea.

The CP content of sample on 1.5% urea treatment was similar to that on 1.0% urea treatment but significantly (P<0.05)higher than those on 0.5 and 2.0% urea treatment (which were similar). The CP content of the control sample was significantly (P<0.05) the lowest. The percentage ether extract of all SBR samples were significantly (P<0.05) higher than the control.

Table 4.2: Proximate composition (%) of urea treated and fermented sorghum beer residue after 4days fermentation

	Levels of urea supplementation											
Parameters (%)*	**0[1]**		**0.5**		**1.0**		**1.5**		**2.0**		**SEM[†]**	
Dry matter,		95.10[a]		94.81[b]		93.99[c]		92.89[d]		92.83[d]		0.22
Crude protein,	21.73[c]		25.19[b]		25.89[ab]		26.45[a]		25.48[b]			0.83
Ether extract,		1.20[c]		2.63[a]		2.39[ab]		2.39[ab]		2.37[b]		0.47
Crude fibre,		14.49[a]		12.11[c]		13.09[b]		12.40[bc]		14.03[a]		0.25
ASH,		24.36[a]		20.54[b]		19.45[b]		24.06[a]		15.92[c]		1.57
NFE,		37.43[c]		39.54[b]		39.29[b]		34.69[d]		42.21[d]		1.25

[a, b, c, d] Means of the same row with different superscripts are significantly different (P<0.05)
*Values represents averages of three determinations per sample
[1]Unfermented sorghum beer residue (control)
[†]Standard error of mean

The percentage ether extract (EE %) of samples on 0.5, 1.0 and 1.5% were similar while those on 1.0, 1.5 and 2.0 were also similar (P>0.05). That on 0.5% urea treatment was significantly (P<0.05) higher than that on 2.0% urea treatment. In terms of crude fibre composition, sample on 2.0% urea treatment was significantly (P<0.05) higher than those on 0.5, 1.0, and 1.5% urea supplementation but similar to the control. Samples on 0.5 and 1.5% urea treatment were similar and there were no significant difference between those on 1.0 and 1.5% urea supplementation. The percentage ash for the control and that of 1.5% urea supplemented SBR sample were similar but significantly (P<0.05) higher than those on 0.5, 1.0, and 2.0% urea treatment. Samples on 0.5 and 1.0 % urea treatments were similar but significantly (P<0.05) higher than the sample on 2.0% urea treatment.

4.1.6. Effect on nutrient composition of methionine treated and fermented sorghum beer residue

Table 4.3 shows the effect on nutrient composition of methionine treated and fermented SBR samples. The DM% composition of the control sample compared to that on 0.41% sulfur treatment was similar but significantly (P<0.05) higher than those on 0.29, 0.33 and 0.39% sulfur treatment which all had similar DM composition. The CP% was observed to increase with increase in the levels of methionine supplementation. The crude Protein content of all samples on 0.29, 0.33, 0.39 and 0.41% methionine treatment did not differ significantly (P>0.05) but were significantly (P<0.05) higher than the control.

Table 4.3. Proximate composition (%) of methionine treated and fermented sorghum beer residue

	Level of sulfur supplementation (%)					
Parameters (%)*	**0[1]**	**0.29**	**0.33**	**0.37**	**0.41**	**SEM[†]**
Dry matter,	91.72[a]	89.66[bc]	89.7[bc]	90.20[b]	91.88[a]	0.47
Crude protein,	21.73[b]	24.91[a]	25.15[a]	25.31[a]	25.56[a] `	0.71
Crude fibre,	14.49[a]	14.73[a]	12.3[bc]	12.89[b]	12.16[c]	0.66
Ether extract,	1.20[d]	3.43[a]	2.97[b]	2.38[c]	2.14[c]	0.38
ASH,	24.36[b]	26.86[a]	22.31[c]	20.61[d]	21.44[cd]	1.30
Nitrogen free extract,	37.43[b]	32.61[d]	37.66[b]	38.89[a]	36.15[c]	1.07

[a, b, c, d] Means of the same row with different superscripts are significantly different (P<0.05)
*Values represents averages of three determinations per sample
[1]Unfermented sorghum beer residue (control)

[†]Standard error of mean

The CF% composition of sample on control were similar to that on 0.29% methionine treatment but significantly (P<0.05) higher than that on 0.33, 0.37, and 0.41% methionine treatments. The percentage ether extract (EE %) of the control sample was significantly the (P<0.05) lowest compared to those samples on 0.29, 0.33, 0.37 and 0.41% methionine treatments. Samples on 0.29% sulfur treatment was significantly (P<0.05) the highest. Samples on 0.33% methionine treatment was significantly (P<0.05) higher than those on 0.37 and 0.41% sulfur treatment but significantly (P<0.05) than 0.29%

In terms of percentage ash, samples on 0.29% treatment was significantly (P<0.05) higher than all the samples on 0.33, 0.37, 0.41% methionine treatment and the control. 0.33, and 0.41% methionine treated samples were similar (P>0.05) while 0.37 and 0.41 % methionine treated samples were similar also.

4.1.7 Effect on nutrient composition of urea and methionine treated and fermented sorghum beer residue

Tables 4.4a and 4.4b shows the effect on nutrient composition of ureaand methionine treated and fermented SBR samples. The percentage DM of sample on N_4S_4 treatment was the highest (P<0.05) compared to the control and all other urea and methionine supplemented samples. Samples on N_1S_2, N_3S_4 and the control were similar (P>0.05) in DM composition but significantly (P<0.05) higher than the rest of urea and methionine treated samples. Samples on N_4S_1, N_2S_2, N_3S_2 and N_3S_4 did not differ but were significantly (P<0.05) higher compared to those on other and methionine treatments. In term of CP content, the increase in CP content (38.19 and 37.37%) were observed to be significantly (P<0.05) highest among samples on N_3S_3 andN_4S_3treatments which were similar (P>0.05) (Table 4.4b). The CP content of sample on N_4S_2 was higher than all the other samples on

urea and methionine treatment. Samples on N_3S_2 and N_1S_4 treatment had similar CP content but significantly ($P<0.05$) highercompared to samples on N_1S_2, N_2S_2, N_1S_3, N_2S_4 and, N_4S_4 treatment which were similar. N_1S_2, N_1S_3, N_2S_3, N_3S_4 and N_4S_4 samples were similar but also significantly ($P<0.05$) higher than the control and samples on N_1S_1, N_2S_2, N_3S_1 and N_4S_1 which were similar. The sample on N_4S_1 treatment showed the lowest ($P<0.05$) percentage CP.

The percentage CF of the control sample was significantly ($P<0.05$) higher than that of all urea and methionine treated fermented samples. The CF of samples on N_4S_4 and $N_2\ S_4$ were similar but significantly ($P<0.05$)higher than all other urea and methionine treated SBR fermented samples. Following this, is the sample onN_4S_3 which showed significantly higher ($P<0.05$) CF, followed by sample on N_3S_3 with significantly ($P<0.05$) higher CF compared to rest of samples on urea and methionine treatment. Samples on N_2S_1, N_3S_1, N_4S_2, N_1S_3 and N_3S_4 had similar ($P>0.05$) CF and significantly ($P<0.05$) higher compared to samples on $N_1\ S_1$, N_4S_1, N_1S_2, N_2S_2 and N_3S_3 treatments which were similar but significantly ($P<0.05$) lowest in CF compared to all the and methionine treated samples.

The ether extract (EE) content of samples on N_2S_3 treatment was significantly ($P<0.05$)higher than those on other urea and methionine treated SBR samples. Samples on N_2S_1, N_3S_1, N_1S_3, N_1S_3, N_2S_4 and N_4S_4 were similar but significantly ($P<0.05$) lower than those on N_3S_2, N_4S_2, N_4S_3 and N_3S_4 which did not show any significant difference in EE content. The control sample showed the least ($P<0.05$) EE% compared to all urea and methionine treated samples.

Table 4.4a. Proximate Composition (%)of urea and methionine supplemented and fermented sorghum beer residue

	levels of urea and methionine supplementation									
Parameters* (%)	**0[1]**	**N_1 S_1**	**N_1S_2**	**N_2S_1**	**N_2S_2**	**N_3S_1**	**N_3S_2**	**N_4S_1**	**N_4S_2**	**SEM[†]**
DM	91.72^{bc}	87.88^{h}	92.04^{b}	91.11^{d}	90.36^{dc}	90.12^{e}	91.55^{c}	91.36^{cd}	87.87^{h}	0.38
CP	18.55^{h}	19.13^{gh}	26.12^{de}	20.19^{g}	27.05^{d}	19.57^{gh}	29.04^{c}	21.73^{f}	31.74^{b}	1.38
CF	14.49^{a}	10.21^{hi}	9.22^{j}	11.13^{f}	10.09^{i}	10.97^{fg}	8.39^{j}	10.06^{i}	10.89^{fg}	0.42
EE	1.20^{h}	1.78^{g}	2.01^{e}	2.16^{c}	2.00^{ef}	2.19^{c}	2.28^{h}	2.05^{de}	2.27^{h}	0.07
ASH	24.36^{a}	7.63^{c}	7.05^{c}	6.09^{d}	7.10^{cd}	6.25^{d}	6.26^{d}	6.38^{d}	6.06^{d}	1.05
NFE,	37.43^{f}	61.27^{ab}	56.05^{c}	60.60^{b}	53.77^{cd}	61.04^{ab}	54.05^{c}	63.29^{a}	49.08^{f}	2.44

[a, b, c, d] Means of the same row with different superscripts are significantly different (P<0.05)
*Values represents averages of three determinations per sample
[1]Unfermented sorghum beer residue (control)
NS = Different levels of urea (N) and methionine (S) supplementation
[†]Standard error of mean

DM = Dry matter, CP = crude protein, CF = Crude Fibre, EE = Ether extract, NFE = Nitrogen free extract

Table 4.4b. Proximate Composition (%) of urea and methionine supplemented and fermented sorghum beer residue

	levels of urea and methionine supplementation								
Parameters* (%)	**N_1S_3**	**N_1S_4**	**N_2S_3**	**N_2S_4**	**N_3S_3**	**N_3S_4**	**N_4S_3**	**N_4S_4**	**SEM†**
DM	89.50^{f}	90.91^{d}	88.89^{g}	90.82^{d}	87.87^{h}	91.66^{bc}	89.50^{f}	93.14^{a}	0 .38
CP	26.05^{de}	25.58^{c}	25.33^{e}	27.03^{d}	38.19^{a}	25.00^{e}	37.37^{a}	25.87^{de}	1.38
CF	10.84^{fg}	12.40^{d}	10.61^{gh}	13.99^{b}	11.93^{e}	11.01^{f}	13.19^{c}	13.86^{b}	0.42
EE	2.15^{c}	1.90^{f}	2.39^{a}	2.12^{cd}	1.91^{f}	2.28^{h}	2.31^{h}	2.15^{c}	0.07
ASH	7.99^{b}	9.02^{b}	6.21^{d}	6.96^{d}	6.11^{d}	6.68^{d}	6.76^{d}	6.39^{d}	1.05
NFE	52.97^{de}	51.08^{ef}	55.48^{c}	49.90^{f}	22.88^{g}	55.04^{c}	40.38^{f}	51.74^{de}	2.44

[a, b, c, d] Means of the same row with different superscripts are significantly different (P<0.05)
*Values represents averages of three determinations per sample
NS = Different levels of urea (N) and methionine(S) supplementation
[1]Unfermented sorghum beer residue (control)
[†]Standard error of mean

The percentage ash for the control sample was significantly ($P<0.05$) higher than that of all the samples on nitrogen plus sulfur treatment. The ash content of samples on N_1S_1, N_1S_2 and N_2S_2 were similar but significantly ($P<0.05$) lower than those on N_1S_3, and N_1S_4 which were similar. The ash content of samples on N_1S_1, N_1S_2 and N_2S_2 were significantly ($P<0.05$) higher than that of samples on N_2S_1, N_3S_1, N_4S1, N_3S_2, N_4S_2, N_3S_3, N_4S_3, N_2S_4, N_3S_4 and N_4S_4 which were all similar ($P>0.05$).

4.1.8. Bacteria population count on wet re-fermented sorghum beer residuesamples onplates

Table 4.5 shows the results of the bacteria count of all fermented wet SBR samples with the corresponding percentage protein biomass produced. The microbial profile produced revealed good effect of fermentation on the initial SBR samples. The bacteria count ranges from 4.0×10^5 (least) to 1.78×10^7 (highest) colony forming unit per gram (cfu/g) in the media.

There were no definite growth pattern observed in bacteria population produced between samples on different substrate to inoculum concentration, different levels of urea and methionine supplementation as well as those on different levels of urea plus methionine supplemented samples. The increased protein biomass produced from fermentation of all SBR samples was not reflected in the corresponding bacteria population revealed (Table 4.5). The highest population growth obtained from the four sets of fermentation were; 6.0×10^6 (without supplementation), 6.0×10^5 (with urea supplementation), 1.32×10^7 (with methionine supplementation) and 2.86×10^6 (with urea plus methionine supplementation). Urea plus methionine treated and fermented sample showed the least bacteria count/ml and the highest percentage crude protein.

Table 4.5. Crude Protein levels of fermentedsorghum beer residue samples and their corresponding bacteria count.

*Sample	Crude Protein (%)	Bacteria Count /ml
SBR	21.73	-
Substrate to inoculum concentration		
10:1	22.10	3.80×10^6
10:2	20.72	6.00×10^6
10:3	19.99	4.70×10^6
10:4	22.41	4.70×10^6
FSBRN		
N_1	25.19	1.56×10^7
N_2	26.89	6.60×10^6
N_3	26.45	1.42×10^7
N_4	25.48	1.78×10^7
FSBRS		
S_1	25.81	6.80×10^6
S_2	24.92	1.32×10^7
S_3	25.15	6.90×10^6
S_4	26.56	5.10×10^6
RSBRN		
N_1S_1	19.13	2.30×10^6
N_2S_1	20.19	1.40×10^6
N_3S_1	19.57	4.00×10^5

N_4S_1	18.55	1.60×10^6
N_1S_2	26.12	2.40×10^6
N_2S_2	27.05	2.50×10^6
N_3S_2	29.04	1.20×10^6
N_4S_2	31.74	2.00×10^6
N_1S_1	26.05	1.30×10^6
N_2S_3	25.33	1.40×10^6
N_3S_3	38.19	6.00×10^5
N_4S_3	37.37	8.00×10^5
N_1S_4	25.58	2.80×10^6
N_2S_4	27.03	1.90×10^6
N_3S_4	25.80	1.40×10^6
N_4S_4	25.87	6.00×10^5

*Results represents averages of three determinations per sample
FSBRN = fermented SBR with urea supplementation
FSBRS = fermented SBR with methionine supplementation

4.2.0. Comparison of the chemical composition and amino acid profiles of SBR and urea, methionine and urea plus methionine fermented SBR samples with highest proteins produced

Table 4.6 shows the average chemical composition and amino acid profiles of SBR analyzed before and after fermentation with urea, methionine, and urea plus methionine supplementation. Compared to the crude protein content of all the fermented SBR samples, the unfermented SBR sample (control) was observed to contain the least crude protein content (21.73%). Fermentation of SBR samples for 4days with urea, methionine, and urea plus methionine supplementation all resulted in increased crude protein content. The CP content of SBR was observed to increase from 21.73% before fermentation to 26.89, 26.56, and 38.19% after fermentation on urea, methionine and urea plus methionine

supplementation respectively (Table 4.6). The highest protein content of 38.19% was obtained with fermented SBR samples with urea plus methionine supplementation (N_3S_3).

The protein biomass produced by samples on methionine supplementation (FSBRS) was observed to be similar to that yielded by SBR samples on urea supplementation FSBRN (Table 4.6). The protein biomass production on methionine supplementation was not in trend with that produced by samples on urea and nitrogen plus sulfur supplementation**.**

The Crude fibre content was observed to decrease from 14.73% before fermentation to 11.93% after fermentation. The CF content of the methionine supplemented SBR sample was observed to be lower than the control but higher than the nitrogen and urea plus methionine supplemented SBR samples. The fermented urea plus v supplemented SBR sample showed the least CF content (11.93%). The level of CF was observed to relate inversely in proportion to the concentration of proteins.

Table 4.6. Proximate composition and amino acid profiles of SBR and highest Protein products of fermented SBR with urea, methionine and urea plus methionine supplementation

Proximate Composition	**SBR**	**FSBRN**	**FSBRS**	**RSBR**	
Dry matter, %		95.10	92.98	91.88	87.87
Crude Protein, %		21.73	26.89	26.56	38.19
Crude Fat %		1.2	2.39	2.14	1.91
Crude fibre, %		14.73	12.4	14.49	11.93
Amino acid composition (g/100g protein)[a]					
Alanine	8.66	7.22	8.51	8.89	

Arginine		4.66	4.57		4.31	5.18
Cysteine		2.18	1.65		1.85	2.38
Glycine	3.17	2.79		2.88	3.60	
Histidine		2.40	1.83		2.21	2.90
Isoleucine		4.18	3.11		3.74	4.63
Leucine	13.71	10.59		13.22	14.31	
Lysine		2.38	2.11		2.32	3.02
Methionine		1.93	1.16		1.88	2.19
Phenylalanine		5.15	4.22		4.98	5.48
Threonine		3.48	3.20		3.21	4.53
Tyrosine		4.13	3.25		3.81	4.44
Serine		4.56	3.8		4.29	4.99
Valine		5.27	4.34		5.04	5.62

[a]values represents averages of two determinations per sample

The amino acid profiles of SBR and all fermented SBR samples revealed the availability of allessential amino acids except tryptophan (alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) in the samples.

The amino acid concentrations (g/100g protein) of all the essential amino acid of the fermented urea plus v SBR sample were higher than those of fermented urea and v treated SBR samples as well as the control. The amino acid concentrations (g/100g protein) of fermented methionine treated sample for the essential amino acids; cysteine, isoleucine, leucine, lysine, methionine, phenylalanine and valine were higher than those of fermented urea treated SBR sample (Table 4.6).

4.7.0 Comparison of the amino acid profile of the protein of urea plus methionine treated and fermented SBR with that of other proteins.

Table 4.7 shows the amino acid composition of the 38.19% total protein biomass of fermented urea plus methionine supplemented SBR sample with the amino acid profile of four other animal feed protein standards. The amino acid profile of the protein biomass produced after 4days fermentation of SBR with urea plus methionine supplementation differ from the amino acid profile of GNC protein. Except for arginine, glycine and valine which were lower, all other essential amino acid concentrations were higher in urea plus methionine supplemented and fermented SBR than those of GNC.The amino acid concentrations of RSBR was numerically higher than that of Soya bean and FAO standards (with the exception of lysine which was lower compared to both) as well as that of Animal Feed Standard. Moreover, it has a superior profile in amino acid concentrations when compared to plant proteins.

Table 4.7. Comparison of the amino acid profile (g/100g protein) of the protein biomass of urea plus methionine treated and fermented sorghum beer residue with that of otherproteins.

Amino acid	*RSBR	GNC	ǂFAO[a]	Soyabean[b]	Animal feed standard[a]

Alanine							
8.89		2.10	-	-		-	
Arginine		5.18		6.35	-	8.3	-
Cysteine		2.38		2.1	2.0	1.0	0.74
Glycine	3.60		7.8	-	4.5	2.43	
Histidine		2.9		2.8	-	3.0	-
Isoleucine		4.63		2.1	4.20	5.6	2.57
Leucine	14.31		9.9	4.80	8.2	3.80	
Lysine		3.02		2.1	4.20	6.8	3.20
Methionine		2.19		0.49	2.20	1.4	0.72
Phenylalanine		5.48		4.3	2.80	4.9	2.20
Threonine		4.53		4.3	2.80	4.0	1.97
Tyrosine		4.44		1.4	-	4.2	-
Serine		4.99		7.8	-	-	-
Valine		5.62		10.0	4.20	5.50	2.70

‡Food and Agricultural Organization
*Urea plus methioninetreated and fermented sorghum beer residue
GNC= Groundnut cake
Sources: a= Araujo D'Souza (1986),
b = Adapted from Allen (1991),
c = Lo and Morean (1986)

4.8.0 On-farm preparation of re-fermented urea plus methionine supplemented Sorghum Beer Residue

The results of the proximate composition carried out after bulk re-fermentation of urea plus methionine supplemented SBR for use in feeding trials are as shown in Table 4.8. The crude protein content of SBR was observed to increase from 24.44% before fermentation to 33.86% after bulk fermentation of urea plus methionine supplemented SBR ($RSBR^2$). The crude fibre content was observed to decrease from 37.90% before fermentation to 13.59% after bulk fermentation.

4.9.0 Effect of feeding graded levels of fermented urea plus methionine supplemented sorghum beer residue on the performance and carcass characteristics of broiler chickens

4.5.1 Starter Period (0-4 weeks)

The performance of broiler chicks on the experimental diets during the starter phase is as shown in Table 4.9. Daily feed intake of birds on 50 and 75% RSBR were similar but significantly (P<0.05) higher than those on control, 25 and 100% RSBR. The daily feed intake of birds on the control and 100% RSBR diets were similar (P>0.05) but significantly (P<0.05) lowerthan those on 25% RSBR. A polynomial trend in feed intake was established as feed intake was observed to increase with increasing level of RSBR from 0 to 75% RSBR beyond which it drops. The final and average daily weight gain for the birds on 25 and 50% RSBR and the control were statistically similar. Daily weight gain of birds on 25 and 50% RSBR were significantly (P<0.05) better than those on 75 and 100% RSBR diets. The daily weight gain of birds on the control and 75% RSBR diets were similar (P>0.05 but significantly higher (P<0.05) than those on

100% RSBR.

Table 4.8. Proximate composition (%) of analyzed sorghum beer residues before and after refermentation

Parameters (%)*	SBR	[1]RSBR	[2] RSBR
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	38.19	33.86

*Values are means of three determinations
[1]RSBR from laboratory study.
[2]RSBR sample from bulk fermentation for feeding trials

Table 4.9: Performance of broiler chicks fed urea plus methionine supplemented and fermented Sorghum beer residue during the starter phase (0-4weeks)

Parameter	Levels of RSBR inclusion (%) 0	25	50	75	100	SEM†
Initial weight (g/bird)	54.10	54.22	54.21	54.12	54.19	0.02
Final weight (g/bird)	844.62^{ab}	876.12^{a}	851.47 ^{a}	790.90^{b}	565.18^{c}	56.85
Weight gain (g/bird)	790.52 ^{ab}	821.90 ^{a}	797.26 ^{a}	736.78 ^{b}	510.99 ^{c}	56.85
Daily weight gain (g/bird)	28.23 ^{ab}	29.35 ^{a}	28.47 ^{a}	26.31 ^{b}	18.25^{c}	2.03
Total feed intake (g/bird)	1340.17 ^{c}	1442.48 ^{b}	1481.69 ^{a}	1499.95 ^{a}	1312.21^{c}	37.81
Daily feed intake (g/bird)	49.64^{c}	53.43 ^{b}	54.88 ^{a}	55.55 ^{a}	48.60 ^{c}	1.40
Feed to gain ratio	1.76^{a}	1.82 ^{ab}	1.92 ^{b}	2.11 ^{c}	2.66^{d}	0.16
Feed cost/kg gain (₦)	176.90 ^{ab}	175.51^{a}	173.46^{a}	184.25^{b}	217.35^{c}	8.19
Mortality (%)	1.39 ^{c}	0.47^{b}	0.0 ^{a}	0.47 ^{b}	0.47^{b}	0.23

[A,b,c] ,Means within rows with different superscripts differ significantly (P<0.05)

RSBR = Urea plus methioninesupplemented and fermented SBR

[†]Standard error of mean (SEM) = S /√n or √ S^2 /√n (Where S = Standard deviation, S^2 = Variance and n = number of treatments)

The feed to gain ratio of birds on 100% RSBR diets was significantly (P<0.05) poorer compared to those of birds on other RSBRand control diets. The feed to gain ratio of birds on 75% RSBR diet was significantly (P<0.05) higher than those on the control, 25 and 50% RSBR diets. The feed to gain ratio of birds on 25% were similar to those on control and 50% RSBRdiets. The feed to gain ratio of birds on 50% RSBR diet was significantly poorer (P<0.05) than those on control. Feed to gain ratio was observed to increase with increasing level of RSBR in the diet.

At the time of the trial, the cost of the various feed ingredients put the cost of a kilogram of the experimental diets (0, 25, 50, 75 and 100%) at ₦104.06, ₦99.72, ₦ 93.26, ₦ 90.32, and ₦84.57 respectively. The cost of a Kg of diet was observed to decrease with increased levels of RSBRinclusion in the diets. The feed cost /kg gain (₦) for the birds on the control and other RSBR diets were ₦176.90, N175.51,₦173.64, ₦184.25, and ₦217.35 respectively. The feed cost of producing a kilogramme of broiler for birds on25 and 50% RSBR diets was between ₦ 1.39 and ₦ 3.44 cheaper than the feed cost of producing a kilogramme of broiler for birds on the control. During the starter phase mortality was significantly (P<0.05) higher for birds on control compared to those on other diets. Mortality was similar (P>0.05) for birds on 25, 75, and 100% RSBR. Mortality was not recorded for birds on 50% RSBR.

4.5.2 Finisher Phase (4-8 weeks)

The results of the performance of broiler birds on the experimental diets during the finisher period are presented in Table 4.10. The final and daily weight gains of birds fed the control and up to 50% RSBR were similar but significantly (P<0.05) better than those fed 75 and 100% RSBR

diets.

Table 4.10: Performance of broiler chicks fed fermented urea plus methionine supplemented SBR during the finisher phase(4-8weeks)

	Levels of RSBR inclusion (%)					
Parameter	**0**	**25**	**50**	**75**	**100**	**SEM†**
Initial weight (g/bird)	844.62[ab]	876.12[a]	851.47 [a]	790.90[b]	565.18[c]	56.85
Final weight (g/bird)	2805[a]	2758 [a]	2835 [a]	2439[b]	1772[c]	200.39
Weight gain (g/bird	1960.38 [a]	1881.88 [a]	1983.53 [a]	1648.1 [b]	1206.82 [c]	145.03
Daily weight gain (g/bird	70.01 [a]	67.21 [a]	70.84 [a]	58.86 [b]	43.10 [c]	5.18
Total feed intake (g/bird)	4555.93 [c]	4442.49 [c]	4882.28 [a]	4632.28 [b]	5031.16 [a]	108.21
Daily feed intake (g/bird)	162.71 [c]	156.66 [c]	174.37 [a]	165.44 [b]	179.68 [a]	4.12
Feed to gain ratio	2.32 [a]	2.33 [a]	2.46 [a]	2.81[a]	4.17[b]	1.98
Feed cost/kg gain (₦)	240.40[a]	241.41[a]	238.40[a]	260.54[a]	362.24[b]	23.54
Mortality (%)	3.54 [c]	3.07 [b]	4.09[d]	1.73[a]	4.13[d]	0.44

Means within rows with different superscripts differ significantly (P<0.05)

RSBR = Fermented urea plus methioninesupplemented SBR

†Standard error of mean (SEM) = S /√n or √ S^2 /√n (Where S = Standard deviation, S^2 = Variance and n = number of treatments)

The daily weight gains of birds on 75% RSBR diet were significantly (P<0.05) better than those fed 100% RSBR diet.Generally, inclusion of RSBR in the diet up to 50% supported better final and daily weight gain.

The daily feed intake of birds on the control and 25% RSBR diets were similar (P>0.05) but significantly lower (P<0.05) than those of birds on other diets. Birds on 50 and 100% RSBR diets had similar daily feed intake which was significantly (P<0.05) higher than those of birds on other diets. Feed intake was generally observed to linearly increase with increased level of RSBR inclusion in the diets. The feed to gain ratio of birds fed 25, 50 and 75% RSBR and the control diets were all similar but significantly (P<0.05) better than those on 100% RSBR diet. Numerically, feed to gain ratio increase with increased level of RSBR.

The cost of producing one kilogramme of live weight of each bird during the finisher phase was observed to be cheaper numerically for birds fed 50% RSBR diet compared to those on control and other diets. The feed cost per kilogramme gain for birds on 100% RSBR diet was significantly (P<0.05) higher compared to those on control, 25, 50 and 75% RSBR diets. The cost of producing one kilogramme of live bird weight increase with increased in RSBR levels in 25,75 and 100% RSBR diets. The diet with 100% RSBR inclusion was most expensive (P< 0.05) in terms of feed cost per kg gain.

Mortality during the finisher phase was similar (P>0.05) for birds on 50 and 100% diets (4.09 and 4.13% respectively) but significantly (P<0.05) higher compared to those for birds on other diets. The lowest mortality rate (1.73%) was observed with birds on 75% of RSBR diet, while bird on 25% RSBR diet had a lower (P<0.05) rate of mortality (3.07%) compared to those on the control diet (3.54%).

4.5.3 Performance of broiler chicks fed fermented urea and methionine supplementedSBR from starter to finisher (0-8weeks)

Observations on the performance of the birds from the commencement to the end of the trial (0-8weeks) presented in Table 4.11 revealed significant (P<0.05)differences in performance parameters across the treatments. Birds fed with the 50 and 100% RSBR diets had similar daily feed consumption but consumed significantly (P<0.05)more feed than those on control and 25% diets. Feed intake and level of RSBR were linearly related.

The daily weight gains for birds on the control, 25 and 50% RSBR diets were similar (P>0.05) but significantly (P<0.05) higher compared to those on 75 and 100%RSBR diets. Weight gain for birds on 75% RSBR diet was significantly (P<0.05) higher than those on 100% RSBR diet. Weight gain was observed to drop significantly when the level of RSBR inclusion in the diets exceeded 50%. The feed to gain ratio of birds on the control, 25 and 50% RSBR diets were similar but significantly (P<0.05) better compared to those birds on 75 and 100% RSBR diets. Thus 75 and 100% RSBR diets were poorly utilized for gain compared to those on control, 25 and 50% RSBR diets. Birds on the 75% RSBR diet had significantly (P<0.05) better feed to gain ratio compared to birds on 100% RBDG diet. Feed efficiency was observed to be consistent among birds on the control, 25 and 50% RSBR diets but dropped beyond 50% level of RSBR inclusion in the diets.During the entire feeding trial (0-8 weeks) percentage mortality was similar between birds on control and 100% RSBR diets and significantly (P<0.05) higher compared to those on other diets. The percentage mortality for birds on 75% RSBRwas significantly the least compared to that of birds on 25 and 50% RSBR diets. The percentage mortality for birds on 25% RSBR diet was significantly (P<0.05) lower compared to those on 50% RSBRdiet.

Table 4.11: Performance of broiler chicks fed fermented urea plus methionine supplemented SBR from starter to finisherPhases (0-8weeks)

	Levels of RSBR inclusion (%)					
Parameter	**0**	**25**	**50**	**75**	**100**	**SEM**
Initial weight (g/bird)	54.10	54.22	54.21	54.12	54.19	0.02
Final weight (g/bird)	2805[a]	2758[a]	2835[a]	2439[b]	1772[c]	200.39
Weight gain (g/bird	2750.9[a]	2703.78[a]	2780.79[a]	2384.88[b]	1717.81[c]	200.39
Daily weight gain (g/bird	49.12[a]	48.28[a]	49.66[a]	42.59[b]	30.68[c]	3.58
Total feed intake (g/bird)	5896.1[c]	5884.97[c]	6363.97[a]	6132.21[b]	6343.37[a]	103.65
Daily feed intake (g/bird)	105.29[c]	105.09[c]	113.64[a]	109.5[b]	113.27[a]	1.85
Feed to gain ratio	2.41[a]	2.19[a]	2.29[a]	2.57[b]	3.69[c]	0.29
Mortality (%)	4.93[d]	3.54[b]	4.09[c]	2.20[a]	4.60[d]	0.48

[a,b,c]Means within rows with different superscripts differ significantly (P<0.05)

RSBR = Fermented urea plus methionine supplemented SBR

SEM = S /√n or √ S^2 /√n (Where S = Standard deviation, S^2 = Variance and n = number of treatments)

4.5.4 Behavior of birds on RSBRdiets

Birds placed on 100% GNC replacement were observed to feed selectively with increased feed wastage between the 14th and 21st days of the starter period. Their faeces were observed to be often wet compared to those on control and other diets.

4.5.5 Feathering of starter birds on RSBR diets

At the end of the brooding period (4-weeks) poor feather development (ruffled) was observed with bird on 100% RSBRdiet compared to birds on other diets. The poor feathering was not observed by the end of the 5th week

4.5.6 Carcass characteristics of finished birds

Table 4.12 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, among birds on 0 and 25% RSBR diets and also among birds on 75 and 100% RSBR diets. The dressing percentage among birds on 50% RSBR diet was highest significantly ($P<0.05$) compared to that of birds on other diets. The dressing percentage of birds on 0 and 25% RSBR diets were significantly ($P<0.05$) higher compared to those on 75 and 100% RSBR diets.

The breast weight percentage for birds on the control was observed to be highest significantly ($P<0.05$) while that for birds on 100% RSBR diet to be the lowest significantly ($P<0.05$). The percentage breast for birds on 50% RSBR diet was significantly higher than those on 25% RSBR

diet and significantly (P<0.05)lower than those of birds on 75% RSBR diet. The thigh percentage of birds on control and 50% RSBR diets were similar but significantly (P<0.05)higher than those of birds on other diets. Birds on 25 and 75% RSBR diets had similar thigh percentage which was significantly

Table 4.12: Carcass characteristics and organ weights of broiler chickens fed urea plus methionine supplemented and fermented SBR in diets (0-8 weeks)

	Levels of RSBR inclusion (%)					
Parameter	**0**	**25**	**50**	**75**	**100**	**SEM**
Final live weight (g/bird)	2803.33[a]	2723.33[a]	2823.33[a]	2436.67[b]	1766.67[c]	193.34
Dressing %	80.3[b]	80.17[b]	82.05[a]	77.99[c]	77.36[c]	0.85
Breast weight (%)	25.03[a]	22.16[c]	23.62[b]	20.48[d]	19.15[e]	1.05
Thigh weight (%)	23.25[a]	21[b]	23.93[a]	20.2[b]	14.57[c]	1.66
Back weight (%)	14.86[b]	14.69[b]	13.64[d]	14.23[c]	15.28[a]	0.28
Wings (%)	7.49[c]	8.51[a]	7.02[d]	8.14[b]	8.77[a]	0.32
Organs						
Liver (%)	2.32[c]	2.63[a]	2.36[c]	2.6[a]	2.55[b]	0.06
Gizzard (%)	2.32[c]	2.26[c]	2.3[c]	2.6[b]	2.92[a]	0.13
Heart (%)	0.48[c]	0.49[c]	0.47[c]	0.55[b]	0.66[a]	0.04
Abdominal fat (%)	2.32[a]	1.53[c]	2.42[a]	1.85[b]	1.79[b]	0.17

Intestinal length (cm)	216[c]	227.67 [b]	229.67 [b]	263.33 [a]	211.33[c]	9.11

Means within rows with different superscripts differ significantly (P<0.05)

RSBR = urea plus methionine supplemented and fermented SBR

(P<0.05) higher than those on 100% RSBR diet. The percentage back of birds on 100% RSBR diet was the highest significantly (P<0.05) compared to those of birds on other diets. Back percentages for birds on control and 25% RSBR diets were similar but significantly (P<0.05) higher than those on 50% and 75% RSBR diets. The percentages for wings of birds on the 25 and 100% RSBR diets were similar and the highest (P < 0.05) compared to those of birds on control and other RSBR diets. The lowest wing percentage was observed with birds on 50% RSBR diet. The wing percentage of birds on 75% RSBR diet was significantly (P<0.05) higher than those on control diet. The percentage liver of birds on 25 and 75% RSBR diets were similar (P>0.05) but significantly (P<0.05) higher than those on 0, 50 and 100% RSBR diets. The percentage liver of birds on 0 and 50% RSBR diets did not differ (P>0.05) but were significantly (P<0.05) lower compared to those on 100% RSBR diet.

The gizzard and heart percentages for birds on 0, 25, and 50% RSBR diets were similar but significantly (P<0.05) lower than those for birds on 75 and 100% RSBR diets. The gizzard and heart percentages for birds on 100% RSBR diet were significantly (P<0.05) higher than those on 75% RSBR diet. The abdominal fat percentage for birds on control and 50% RSBR diets were similar but significantly (P<0.05) higher than those on 75 and 100% RSBR diets (which were similar.) The abdominal fat percentage for birds on 25% RSBR diet were significantly (P<0.05) the lowest. The intestinal length for birds on 75% RSBR diet was the highest (P<0.05) while the intestinal length of birds on 25 and 50% RSBR diets were similar (P>0.05) but significantly (P<0.05) higher than those on control and 100% RSBR diets which were similar (P>0.05).

4.5.7 Haematological parameters of broiler chickens fed urea plus methionine supplemented and fermented SBR in diets (0-8 weeks)

Observations of the haematological characteristics of broiler chickens fed urea and methionine supplemented and fermented SBR diets from the commencement to the end of the trial (0-8 weeks) presented in Table 4.13 revealed significant (P<0.05) differences in haematological parameters across the treatments, indicating that RSBR diets influenced the values of the parameters. In terms of parked cell volume (PCV), the values for birds on all RSBR supplemented diets ranges from 23.50.00 to 28.33. The PVC values for birds on control, 50 and 100% RSBR diets were similar (P>0.05) but significantly (P<0.05) lower than those of birds on 25 and 75% RSBR diets.Birds on 75% RSBRdiet showed the highest (P<0.05) value compared to those on other diets. The results of the haemoglobin concentration obtained followed a similar pattern with that of PCV. The values for haemoglobin were; 7.15, 8.63, 7.50, 9.43 and 7.63 for 0, 25, 50, 75 and 100% respectively. The haemoglobin concentration (g/dl) among the birds across the treatments was highest (P<0.05) for birds on 75% RSBRdiet and least (P<0.05) among birds on the control, 50 and 100% diets which were similar (P>0.05). The Hb concentration for birds on 75 RSBRdiet showed the highest (P<0.05) values. The haemoglobin concentration for birds on all diets were within the normal range.

Table 4.13 showed that the average amount of total protein (TP) values (g/dl) for birds across the treatments was observed to be similar (P>0.05) for that of birds groups which were fed control, 25 and 50% RSBR diets , and significantly (P<0.05) higher than that for birds on 75 and 100% RSBR diets. The TP value for birds on 100% RSBR diet showed the least (P<0.05). Birds on control, 25 and 50% RSBR diets showed adequate level of proteins while birds on 75 and 100 % RSBR diets showed low level of proteins.

Table 4.13: Haematological parameters of broiler chickens fed urea plus methionine supplemented and fermented SBR in diets (0-8 weeks)

		Levels of RSBR inclusion (%)					
Parameter	**NR**	**0**	**25**	**50**	**75**	**100**	**SEM**
Parked cell volume (%)	(24 – 48)	23.55[c]	26.00[b]	24.00[c]	28.33[a]	23.50[c]	1.52
Haemoglobin (g/dl)	(8.0 -16.0)	7.51[c]	8.63[b]	7.50[c]	9.43[a]	7.63[c]	0.51
Total protein (g/dl)	(4.0 -7.4)	3.47[a]	3.47[a]	3.6[a]	3.06[b]	2.67[c]	0.16

[a,b,v]Means within rows with different superscripts differ significantly (P<0.05)
RSBR = urea plus methionine supplemented and fermented SBR
NR= Normal range (Morton 1994)

SEM = Standard error of mean

4.10.0 Performance of broiler chickens fed 75% urea and methionine treated and fermented sorghum beer residue diets supplemented with specific multi-enzymes.

4.6.1 Physical observation

During the 56days (4 weeks) starter period, birds group on un-supplemented diet were observed to often passed out wet droppings compared to groups on control and enzymes supplemented diets. Uniform and faster growth (based on weight gain) was observed amongst birds on control and enzymes supplemented diets compared to those on 75% RSBR diet. The enzymes supplemented diets werealso observed to have positive effects on the general wellbeing of birds which reduces the need for frequent prophylactic administration of antibiotics.

4.6.2 Broiler starter phase (0-4 weeks)

The results of the performance of broiler chicks fed the control (0% RSBR) and 75% RSBR or the supplemented (75% RSBR + N, 75% RSBR + Z and 75% RSBR + F) diets during the starter phase are as shown in Table 4.14. The final and daily weight gains of birds on +N diet and those on +Z diet were observed to be similar ($P>0.05$) but significantly ($P<0.05$)higher *when* compared to those of birds on control, 75%RSBR and + F diets. The daily weight gain of birds on control, 75%RSBR and + F diets were similar ($P>0.05$). The total and daily feed intake of birds on 75%RSBR, +N and +Z diets were similar but significantly ($P<0.05$) higher than those of birds on control and +F diet. The total and daily feed intake of birds on the control diet was observed to be significantly ($P<0.05$)lower than those on +F diet. The total and daily feed intake of birds on 75% RSBR,+N and +Z diets were similar but significantly ($P<0.05$) higher than those of birds on

control and 75%RSBR +F diets.

Table 4.14: Performance of broiler chicks fed urea and methionine treated and fermented sorghum beer residue supplemented with enzymes during the Starter period (0-4weeks)

Parameter	Levels of RSBR inclusion					SEM*
	0%	75%	75% +Nutriad	75% +Zymos-N	75% +Fullzyme	
Initial weight (g/bird)	42.56	42.77	42.96	42.70	42.95	0.08
Final weight (g/bird)	955.56^{b}	941.77^{b}	1013.00^{a}	1006.66^{a}	945.46^{b}	15.44
Final weight gain (g/bird	913.00^{b}	898.50^{b}	970.04^{a}	963.96^{a}	902.51^{b}	15.48
Daily weight gain (g/bird	32.61^{b}	32.10^{b}	34.64^{a}	34.43^{a}	32.23^{b}	0.55
Total feed intake (g/bird)	1482.60^{c}	1832.88^{a}	1779.96^{a}	1781.36^{a}	1689.52^{b}	62.11
Daily feed intake (g/bird)	52.95^{c}	65.46^{a}	63.57^{a}	63.62^{a}	60.34^{b}	2.22
Feed to gain ratio	1.62^{a}	2.04^{c}	1.84^{b}	1.85^{b}	1.87^{c}	0.07
Feed cost/kg gain (₦)	150.32^{c}	152.20^{c}	139.12^{b}	142.43^{b}	137.04^{a}	3.01
Mortality (%)	1.52^{b}	3.03^{c}	0.0^{a}	1.52^{b}	0.00^{a}	0.57

[a,b,c]Means within rows with different superscripts differ significantly (P<0.05)

RSBR = Fermented urea plus methionine supplemented SBR, N = Nutriad, Z = Zymos-N, F = Fullzyme

*Standard error of mean (SEM) = S /√n or √ S^2 /√n (Where S = Standard deviation, S^2 = Variance and n = number of treatments

The feed to gain ratio of birds on control diet was significantly(P<0.05) the lowest (best) among birds on all treatments while that for birds on 75%RSBR diet showed the highest (poorest) significantly (P<0.05).

The cost of feed per kg gain (₦) for birds on the control and un-supplemented diet were similar but significantly (P<0.05) higher than those on +N, +Z and +F diets. The cost of feed per kg gain (₦) for birds on +F diet was significantly (P<0.05) lowest amongst all treatments. The feed cost per kg gain of birds on +N and +Z diets were similar (P> 0.05). The mortality rate was observed to be similar (P>0.05) among birds on control and +F diet. The rate of mortality was highest (P<0.05) for birds on the 75%RSBR diet. There was no mortality recorded among birds on +N and +F diets during the starter period.

4.6.3 Broiler finisher phase (5-8 weeks)

The results of the performance of broilers fed the control, 75% RSBR with and without enzymes supplemented diets during the finisher phase are presented in Table 4.15. Dietary treatments during the finisher phase had significant (P<0.05) effect on final weights, final weight gain, daily weight gain, feed intake, feed to gain ratio, feed cost per kg gain and on mortality. The final weight and daily weight gains of birds on + N diet was significantly (P<0.05) higher than those on other diets. The final weight and daily weight gains of birds on 75%RSBR diet were significantly (P<0.05) lowest compared to those on other treatments. The total and daily feed intake of birds on +N and +Z diets were similar but significantly (P<0.05) higher than those of birds on control, 75%RSBRand +F diets. Birds on 75%RSBR and +F diets had similar total and daily feed intake, while those on control had the lowest (P<0.05) significantly.

Table 4.15: Performance of broiler chicks fed urea and methionine treated and fermented sorghum beer residue supplemented with enzymes during the finisher phase(5-8weeks)

Parameter	Levels of RSBR inclusion					SEM*
	0%	75%	75% +Nutriad	75% +Zymos-N	75% +Fullzyme	
Initial weight (g/bird)	955.00	955.33	960.41	954.85	964.05	1.85
Final weight (g/bird)	2991.69^{b}	2705.26^{c}	3086.27^{a}	2955.56^{b}	2964.90^{b}	63.62
Final weight gain (g/bird)	2036.67^{b}	1749.93^{c}	2125.86^{a}	2000.71^{b}	2000.85^{b}	62.55
Daily weight gain (g/bird)	72.74^{b}	62.50^{c}	75.92^{a}	71.45^{b}	71.46^{b}	2.23
Total feed intake (g/bird)	3460.86^{c}	3490.39^{b}	3509.07^{a}	3507.04^{a}	3487.64^{b}	8.67
Daily feed intake (g/bird)	123.60^{c}	124.66^{b}	125.32^{a}	125.25^{a}	124.56^{b}	0.31
Feed to gain ratio	1.70^{b}	2.00^{c}	1.65^{a}	1.75^{b}	1.74^{b}	0.06
Feed cost/kg gain (₦)	148.10^{c}	151.48^{c}	126.24^{a}	136.71^{b}	135.18^{b}	4.58
Mortality (%)						
3.33^{c} 5.00^{d}	0.00^{a}	1.67^{b}	0.00^{a}			0.97

^{a,b,c}Means within rows with different superscripts differ significantly (P<0.05)

RSBR = Fermented urea plus methionine supplemented SBR

*SEM = S /√n or √ S^2 /√n (Where S = Standard deviation, S^2 = Variance and n = number of treatments)

The feed to gain ratio of birds on +N diet was better (P<0.05) compared to those on control and other diets. The feed conversion efficiency of birds on 75%RSBR diet was significantly (P<0.05) lower than those on control and +Z diet which were similar (P>0.05). There was no significant (P>0.05) difference in feed cost per kg gain for birds on control diet and those on 75%RSBR diet, but they were significantly (P<0.05) more expensive compared to diets of other birds. Feed cost/kg gain for birds on +N diet was significantly (P<0.05) lowerthan those of +Z and +F diets which were similar (P>0.05).

There were no record of mortality for birds on +N and +F diets during the finisher phase. The mortality rate for birds on the 75%RSBR diet was significantly higher than those on control and +Z diets. The rate of mortality of birds on the control diet was significantly higher (P<0.05) than that of birds on +Z diet.

4.6.4 Carcass characteristics

Table 4.16 shows the results of carcass analysis of the experimental bird. The dressing percentage for birds on +N diet was significantly (P<0.05) higher than those of birds on control and other diets. The dressing percentages for birds on +Z and +F diets were similar (P>0.05) but significantly (P<0.05) higher than those on control and 75%RSBR diets. Birds on 75%RSBR diet showed the lowest (P<0.05) dressing percentage compared to those on the control diet. The dressing percentage of birds on control and +Z diets were similar (P>0.05).

Table 4.16: Carcass characteristics and organ weights of broiler chickens fed urea plus methionine treated and fermentedSorghum beer residue supplemented with multi-enzymes

Parameter	Levels of RSBR inclusion					SEM*
	0%	75%	75% +Nutriad	75% +Zymos-N	75% +Fullzyme	
Final live weight (g/bird)	3013.33^{b}	2755.25^{c}	3180.87^{a}	2992.55^{b}	3000.11^{b}	67.89
Dressing %	75.22^{c}	73.80^{d}	80.00^{a}	75.74^{bc}	76.50^{b}	1.20
Breast weight (%)	22.29^{b}	21.69^{c}	24.44^{a}	22.72^{b}	24.22^{a}	0.54
Thigh weight (%)	21.75^{b}	20.96^{c}	22.38^{a}	21.17^{b}	21.89^{b}	0.26
Back weight (%)	15.70^{a}	14.64^{c}	14.99^{b}	14.70^{c}	14.61^{c}	0.20
Wings (%)	8.31^{c}	7.97^{d}	9.26^{a}	8.47^{c}	8.78^{b}	0.22
Liver (%)	2.05^{b}	2.50^{a}	2.04^{b}	2.23^{ab}	2.42^{ab}	0.30
Gizzard (%)	2.10^{d}	3.00^{a}	2.46^{c}	2.56^{c}	2.78^{b}	0.15
Heart (%)	0.66^{b}	0.73^{a}	0.58^{c}	0.56^{c}	0.67^{b}	0.03
Abdominal fat (%)	2.21^{d}	2.78^{a}	2.52^{b}	2.40^{c}	2.56^{b}	0.09
Intestinal length (cm)	234.33^{c}	254.00^{a}	245.00^{b}	232.67^{c}	226.00^{d}	4.95

^{a,b,c,d}Means within rows with different superscripts differ significantly (P<0.05)

RSBR	=	Fermented	ureaandmethionine	supplemented	SBR

The percentage breast for birds on +N and +F diets were similar and significantly (P<0.05)higher than those on control, 75%RSBR and +Z diets. Birds on control and +Z diets had similar percentage breast (P>0.05), while birds on 75%RSBR diet showed the lowest percentage breast (P<0.05) compared to those on other diets. In terms of thigh weight percentages, significant (P>0.05)differences were not observed for birds on the control and +Z and +F diets. Birds on +N diet showed the highest (P<0.05) thigh weight percentage while birds on 75%RSBR diet showed the lowest (P<0.05) thigh weight percentage. Percentage back weight for birds on 75%RSBR, +Z and +F diets were similar (P>0.05), but significantly(P<0.05) lower than the percentage back weight for birds on +Z diet. Percentage back weight for birds on control diet was the highest significantly compared to those of birds on other diets.

The wings weight percentage for birds on +N diet was significantly the highest (P<0.05) while that for birds on 75%RSBR diet showed the lowest (P<0.05). The wings weight percentage for birds on control and +Z diets were similar (P>0.05) but significantly lower than those on +F diet. Birds on 75%RSBRdiet had numerically the highest liver percentage compared to birds on other diets. Percentage liver weight for birds on 75%RSBR diet was significantly higher (P<0.05) than those on the control and +N diets. Significant differences (P<0.05) were not observed for percentage liver for birds on +N, +Z, +F and the control diets. The percentage gizzard was observed to be similar between birds on +N and +Z diets and significantly lower when compared to that of birds on +F diet. Birds on control diet showed the lowest (P<0.05) percentage gizzard while those on 75%RSBR diet showed the highest (P<0.05) percentage gizzard. Percentage heart for birds on 75%RSBR diet was significantly the highest compared to those on control and other diets. The percentage heart for birds on control and +F diets were similar (P>0.05) but significantly (P<0.05)higher when compared to those of birds on +N and +Z diets which were

similar.

There were significant ($P<0.05$) differences in percentage abdominal fat among birds on the different diets.

There were no significant difference ($P>0.05$) in percentage abdominal fat between birds on +N and +F diets which was significantly ($P<0.05$) higher than that for birds on +Z diet. Birds on 75%RSBR diet had significantly ($P<0.05$) the highest percent abdominal fat while those on the control diet had the lowest percent abdominal fat ($P<0.050$).

The intestinal length of birds on the control and +Z diets were similar but significantly ($P<0.05$) lower than those on +N diet.Birds on 75%RSBR diet had the longest ($P<0.05$) intestinal length while birds on +F diet showed the shortest intestinal length ($P<0.05$).

CHAPTERFIVE

5.0 DISCUSSION

5.1.0 Effect of different inoculum concentration on nutrient composition of SBR on 4 days fermentation.

The 2nd stage fermentation process of SBR with nitrogen plus sulfur supplementation causes its fibrous nature to become smoother and change of colour to dark brown with the resultant sweetness in odour. Researchers have earlier established these characteristics after fermentation. According to Homb (1984), chopped straw soaked (Beckman soaking method) in NaOH solution resulted in good aroma with increased digestibility and palatability particularly that of CP and CF, while Wilkinson (1984) observed changes associated with ensiled materials after treatment with NaOH to include: heat production, loss of structural rigidity and colour change to deep yellow or yellow –brown. Zandrazil (1984) reported the decomposition of lignocellulosic materials into feed by brown rot fungi at extremely low pH (3.0-2.0). The author observed that as a result, the decomposed material becomes brown due to delignification.

caused significant decrease in the dry matter percentage (DM). This findings were in agreement with Ndams, (2008) and Odufa (1987) who both reported a reduction in DM percentage during fermentation. The increased percentage CP and decreased percentage CF of SBR after 4days fermentation respectively in this study were in agreement with Ndams *et al.* (2009) and Adeyemi and Adeleke (2000). Who reported an increase in %CP of re-fermented brewers dried grains (RBDG) and thevetia cake after fermentation respectively. Similarly, Ofuya and Obilro (1993) after 20 days fermentation of cassava peel reported an increase in %CP value. The use of rumen liquor as inoculum for the fermentation of SBR resulted in significant increase in the CP (0.60%) content from 21.73 to 22.41%. This rate of increase was lower than the 11% increase observed by Ndams, (2008) and 7.74% increase observed by Oyegbile (1988). This differences in the values may be attributed to the differences in fermentation conditions.

5.2.0 Effect on nutrient composition of SBR with urea and methionine treatment after 4days fermentation

The fermentation of SBR treated with different levels of urea resulted in significant increase in the CP content from 21.73 to a peak of 26.45%.This happened because urea supplementation offers alternative source of nitrogen for protein synthesis by fibre digesting bacteria. This result agrees with the affirmation of Kononoff *et al*. (2006) that rumen microbes have a unique abilities of growing on sample containing fractions of feeds. The increase in CP content is in

agreement with the report of Rhodesand Broderick (1989) who carried out a 10-day fermentation of nitrogen supplemented wheat pollard and observed increase in %CP. Similarly, Yalemstesfa *et al.* (2010) obtained a maximum production of 31.73% protein enriched biomass from nitrogen supplemented orange waste used as substrate for the fermentation process using *Aspergillus niger* as inoculum. Raising the nitrogen content of the diet of ruminants was reported to increase the digestibility and intake of the diet (roughage) owing to stimulation of bacteria activity (McDonald *et al*., 1998)

In this study the %CP was observed to increase with increasing levels of urea in the samples but dropped beyond the 1.5% urea supplementation. The 1.5% level of urea might indicate the peak level of ammonia produced as identified by Van Soest (1994). He stressed that urea is rapidly hydrolyzed to ammonia. The drop in %CP beyond 1.5% level could be attributed to ammonia toxicity to the microbes. Straka *et al.* (2007) reported the inhibition of anaerobic fermentation when ammonium nitrogen exceeded 1.2g N/l. According to the researchers, the rate of ammonia concentration is dependent on the content of nitrogen in the substrate, and the utilization of nitrogen is dependent on its ratio with carbon (N/C). Hoover and Stokes (1991) listed factors that may influence nitrogen utilization to include dietary nitrogen, dietary sulfur, and amount of urea, digestible carbohydrate availability, and length of trial. In this study the increase in urea levels without corresponding increase in digestible carbohydrate resulted in increased ammonia concentrations which influenced anaerobic fermentation negatively and thus the drop in percentage CP.

On methionine supplementation CP increases from 21.73% before fermentation to a peak of 25.56% after fermentation. The increase in %CP of SBR samples with different levels of methionine supplementation in this study over the control is in agreement with the report of Stevani *et al.* (1992) that rumen bacteria utilized sulphur amino acids for bacteria protein synthesis. Sulphur is said to be an essential micro-mineral essential for the formation of sulphur containing compounds in the body (Bal and Ozturk, 2006). Studies have shown that sulphur supplementation has improved gain by 12% in steer grazing sorghum-sudan grass containing 0.08-0.12% sulfur (Anonymous, 1998). The similarity in %CP for all fermented methionine supplemented samples are in agreement with the findings of Brondani *et al*. (1991), that the effect of sulfur supplementation on bacteria protein synthesis from high fibre nitrogen based diets will depend on its adjustment based on the N: S ratio. If the level of nitrogen in the diet remain low, the effect of increasing levels of S on bacteria protein synthesis will remain insignificant.

5.3.0 Effect on nutrient composition of urea plus methionine supplemented and fermentedSBR

The fermentation of SBR sample with ureaandmethionine supplementation at a ratio of 1.85 to 5.0% resulted in a significant increase in CP content from 21.73 to 38.19%.

The CP value of 38.19 was higher than the values of 16.9, 34.0, 35.57, 36.0 and 36.41% reported by Ofuya and Obilor (1993), Almeida *et al.*(1995), Isikwenu *et al.* (2007), Ndams, (1991) and Ndams, (2008) respectively. It was also observed to be similar to 38.52% reported by Isikwenu *et al.* (2008). This rate of increase of CP content observed is also in agreement with Brondani *et al*. (1991) who concluded that high fibre diets low in ureawere better utilized by rumen microbes when urea and methionine are adequately supplemented. Nitrogen and sulfur according to

Brondani *et al*. (1991)were precursors that must be adequately made available to rumen bacteria for maximal protein synthesis.

The role of sulphur utilization in nitrogen based diets has been considered for bacterial protein synthesis in terms of N: S ratio. In this study, the N: S (N_3S_3) of 1.5:0.37% seemed to be the adequate levels of nitrogen plus sulphur levels required for optimum bacterial growth and protein synthesis and differs from the typical nitrogen to sulphur ratio of a complete diet of 10:1 advocated (Anonymous, 1998). Fermentation of SBR with N and S supplementation does not only increase the quantity of protein but also confirm the fact that proteins supplied to ruminants are high quality proteins

Fazaeli *et al*. (2007), reported an optimum level of 0.33% sulphur and 0.5% urea for rumen fermentation of roughage based diets in buffalo. Brondani *et al.*(1991) did not observed any change in rumen acetate production when sugarcane bagasse was fed with separate nitrogen and sulfur supplementation, however, when both urea and sulphur were supplemented together, rumen acetate production was 44% higher. Zinn *et al*. (1997) stressed that it was essential to provide an optimum nitrogen-sulphur balance for rumen microbial growth when supplementing sulfur in roughages which are mostly poor in available nitrogen and sulphur. It has been affirmed that in wine making, fermentation without adequate nitrogen will leave one with reduced sulfur compounds and off-smells in the finished products and sulfur supplementation has been advocated when urea or other non-protein nitrogen sources were fed to ruminants (Anonymous, 1998).

5.4.0 Comparison of the amino acid profiles of SBR, FSBRN, FSBRS and RSBR

The availability of essential amino acids in the unfermented (SBR) and in all fermented samples (FSBRN, FSBRSand RSBR) is an indication that the fermented SBR samples can serve as protein sources in monogastric nutrition (McDonald *et al*., 1998). The amino acid content of all the essential amino acids of the fermented nitrogen plus sulphur supplemented SBR samples were numerically lower compared to the unfermented sample in this study. This agreed with Spear *et al*., (1978) who observed numerical decrease in essential amino acids in lambs fed tall fescue supplemented with l-methionine sulphur at 0.05 and 0.15% compared to the control (0%). The amino acid profile of RSBR samples were superior to the control (SBR), FSBRN and FSBRS samples.

5.5.0 Comparison of the amino acid profile of RSBRwith that of other Protein Standards.

The amino acid profile of the protein biomass (RSBR) produced in this study, was observed to be higher numerically than those of GNC. It was also superior to the amino acid profile of other plant proteins. The quality of the RSBR protein produced was equal to or may exceed that of the conventional ingredients in terms of its amino acid profile and the standard set for animal feed. This revealed that RSBR has all the essential amino acids for animal feed. This is in agreement with Ofuya and Obilor (1993) who observed that *Rhizopus* fermented cassava peel feedstuff contained all the essential amino acids that were far more digestible than the control. Also Almeida *et al*. (1995) observed that the protein obtained (34% of the biomass) contained all the

essential amino acids for animal feeds when eucalyptus hydrolyzate was fermented using *paecilomycestes variottii* (fungus).

When compared to the FAO and soya bean standards, one can see that the protein standards and, the protein biomass produced after 4days fermentation of SBR with nitrogen plus sulfur supplementation contains all the essential amino acids for animal feeds. Moreover, it has a superior amino acid profile when compared to other plant proteins.

5.6.0 Effect of feeding graded levels of RSBRon performance and carcass characteristics of broiler chickens

The daily weight gain of broilers at 4-weeks of age showed that the diets with 0, 25, and 50% inclusions were similar, indicating that starter chicks can tolerate inclusion of fermented RSBR up to 50%, which is about 21.35% of the diet, This result exceeded the 30% inclusion level previously achieved (Ndams,2008) with re-fermented brewers dried grains (RBDG) in broiler chick diets but in agreement with the findings of Isikwenu *et al*(2008) who also observed similar daily weight gain between birds fed 0, 25 and 50% urea fermented BDG in place of GNC. Thus the higher inclusion level (50% RSBR) achieved in this experiment can be attributed to the fermentation of SBR with ureaand methionine which resulted in fibre breakdown and reduction in fibre content, and resultant production of increased proteins in quantity and quality which encouraged a good performance of broiler chicks even when fed a diet with higher inclusion level of 21.35% RSBR. The performance may also be as a result of a better complementary role

of nutrients (1:3 and 1:1 ratios) at this level of RSBRto GNC mix as also suggested by Isikwenu *et al.* (2008).

It has been reported that fermentation produces vitamin B-complex which increased appetite for feeds (Lawal *et al.,*2007). This might encourage better nutrients released and availability for enhanced tissue synthesis, leading to improved growth rate, a kind of synergistic effect in chicks. A combination of plant proteins such as grains with pulses led to a high quality protein which was just as good as protein from animal feedstuffs. The daily weight gain by birds on 75% RSBRwas comparable to the control indicating that starter chicks can tolerate inclusion of RSBRup to 75% which was about 34.9% of the diet. The observed numerical increase in daily weight gains of birds on 25 and 50% RSBRdiets over those on the control could be attributed to the superior nutrients furnished by RSBRdiets for tissue synthesis to record enhanced growth rate than the control diet that does not have any RSBR. In addition, the starter birds at this age had the ability to handle the fibre content of the diets (Uchegbu and Udedibie, 1998, Atteh and Opawaude, 2000). The lower weight gain of chicks fed 100% inclusion of RSBR may have resulted from nutrient intake restriction precipitated by lower intake or nutrient dilution effect of high crude fibre and bulkiness of feeds. Similar results were reported on the implication of restricted feed intake on growth rate and body weight performance by Mench (2002) and Erakpotobor and Umeh (2005). Broiler chicks fed RSBRat 50, and 75% inclusion levels had significantly higher feed intake than those on 0, 25, and 100% inclusions levels which means that 50 and 75% inclusion levels were equally acceptable to broiler chicks as they ate approximately the same quantity. However, the decrease in feed intake at the 100% RSBR inclusion levels may be attributed to bulkiness and probably a problem of acceptability of the feed associated with high RSBR levels compared to GNC.

The feed to gain ratio of birds on RSBR on 100% RSBR inclusion level was significantly higher compared to those of birds on other diets. This showed that 100% RSBR diet was least utilized for tissue synthesis as is shown by depressed growth rate compared to birds on other diets. Feed to gain ratio was observed to increase with increased levels of RSBR in the diets. The decrease in efficiency of feed utilization observed in the study as the RSBR levels increased is 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 levels of sorghum distillers' waste inclusion increased in the diet. However, this did not agree with the findings of Yakuugh *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 low mortality observed during the starter phase on all RSBR supplemented diets attested to its suitability and safety as a feed ingredient and as a possible substitute for GNC. The 1.39% mortality recorded for birds on the control which was significantly higher compared to that recorded for birds on RSBR diets occurred as a resultof rainstorm that affected the pen containing birds on control diet, and inadequate heat supply from lanterns and charcoal coupled with "epileptic" electric power supply needed to keep the birds warm led to the loss of 1% of the birds.

The inclusion of RSBR in the diets up to 100% resulted in lower feed cost (₦/kg) when compared to the diet with 100% GNC. It was observed that feed cost of producing a kilogram of broiler on25% and 50% RSBR diets were ₦1.39 and ₦3.44 cheaper than the feed cost of producing a kilogramme of broiler on GNC diets. This was beneficial because on a large scale broiler

enterprise, a savings of one naira (₦ 1.00) in feed cost in producing a kilogramme of broiler is quite significant.

At the end of the finisher period, the daily weight gains of birds on 25 and 50% RSBR diets were similar to those on control, indicating that finisher birds can tolerate inclusions of RSBR up to 50% which was about 17.65% of the diets. The inclusion levels of 25 and 50% were able to furnished adequate nutrients for tissue synthesis to record an enhanced growth rate comparable to the control diet in addition to the ability of the birds at this age to tolerate the fibre content of the diets (Uchegbu and Udedibie; 1998; Atteh and Opawaude, 2000). The least weight gain observed with birds fed the 100% inclusion level may have resulted from nutrient intake restriction or dilution effect of crude fibre and bulkiness of feeds. Jegede (1990) observed that high fibre basal diets carry low proportion of nutrients per unit volume rather than per unit weight. Thus the substitution of high fibre feeds on a weight for weight basis resulted in reduced nutrient intake by the birds. Birds fed RSBR at 50, 75 and 100% inclusion levels had significantly higher feed intake (FI) than those on control and 25% inclusion levels which indicated that 50, 75 and 100% levels of inclusion were well accepted by the birds. The improvement in FI over the control was due to improvement in palatability, texture and digestibility in RSBR by fermentation (Faniyi, 2006). Besides, there was addition of vitamin B-complex during fermentation by rumen organisms which increases the appetite for food in the birds just as vitamin B-complex does for humans (Faniyi, 2006).

The feed to gain ratio (F/G) of birds on control and diets containing 25, 50 and 75% RSBR were similar, which indicated that the efficiency by which the birds utilized the diets for tissue synthesis was the same as control, 25, 50 and 75% RSBR diets. It indicatedsupply of adequate nutrients to support adequate live weight gain. The F/G of birds on 100% RSBR was significantly

higher than those of birds on control and other diets. This showed that 100% RSBR diet was poorly utilized. This confirmed the poor daily weight gains of birds fed 100% RSBR compared to birds fed on control and other diets. This is in agreement with the findings of Isikwenu *et al* (2007) and (2008).

The inclusion of RSBR in finisher diets resulted in lower feed cost per gain (₦/kg gain) of all the diets compared to the control. Diets of 25, 50, 75 and 100% RSBR inclusion were ₦ 0.01, ₦6.71, ₦10.90 and ₦ 16.73 cheaper than 100% GNC (0% RSBR) diet. In terms of live production performances 100% RSBR diet also showed the least. This is in agreement with the assertion of Lemme and Petri (2002) that lowest feed cost per tonne may not likely translate to the best live production performances. It was observed that feed cost of producing a kilogramme of broiler on RSBR finisher diets was only cheaper with birds on 50% compared to the feed cost of producing a kilogramme of broiler on GNC diet. It was expensive to produce a kilogramme of broiler on 25, 75 and 100% RSBR compared to that for birds on 100% GNC diet (control). The 16.56% total mortality rate recorded during the finisher phase was as a result of chronic respiratory disease (CRD) that was initially diagnosed ascoccidiosis. Before the right drugs were administered to contain the problem, 16.00% of the 16.56% overall mortality that occurred during this period had been recorded. The incident occurred by the end of 5^{th} week of the experiment. The mortality recorded throughout the finisher phase was not as a result of dietary treatments. The inclusion of RSBR levels in the diets of birds beyond 25% was observed to cause increased feed consumption. This indicates the increase in acceptability of the diets with increased levels of RSBR in the diets. Faniyi (2006) has established that fermentation brings about improvement in palatability and digestibility with the addition of vitamin B-complex which increases the appetite for feed in birds. This findings was in contrast with the report of Isikwenu

et al. (2007) and Atteh and Opawaude, (2000) who both observed decrease in feed intake with increased levels of Brewers dried grains (BDG) in the diets of broiler chickens.

At the end of the feeding trial (8-weeks), birds on control and on diets up to 75% RSBR inclusions were observed to have attained the average weight of two kilogramme (2kg). According to Barry (2006), a modern broiler chick gains over 43 times its initial weight in an eight week period. This means that the control diet and diets up to 75% RSBR inclusion levels have all furnished adequate nutrients for an enhanced growth by birds on the diets. The feed to gain ratio (F/G) of birds on control, 25 and 50% RSBR diets were similar but better than those of birds on 75 and 100% RSBR diets. meaning that the efficiency at which the birds utilizes the control, 25 and 50% RSBRdiets for tissue synthesis is the same but better than that of birds on 75 and 100% RSBR diets. The F/G ratio of birds on 75 and 100% RSBR diets revealed why the daily gains of birds on 75%RSBR diet were superior compared to those on 100% RSBR diet. It means they were supplying adequate nutrients to support adequate live weight gain. The F/G of birds on 75 and100% RSBR diets was a reflection of the daily gains of birds on 75 and 100% RSBRdiets that was not comparable to those of birds on control, 25 and 50% RSBR Diets. This is in agreement with the findings of Isikwenu *et al*. (2007). The general increase in F/G ratio with increased in RSBR in the diets observed at the end of the trial is in agreement with the previous findings when fermented brewers' dried grains were fed to broiler chickens (Ndams,*et al.* 2009).

The efficiency by which the birds utilizes the diets for tissue synthesis was the same for control, 25, 50 and 75% RSBR diets. It means they were supplying adequate nutrients to support adequate live weight gain. The F/G of birds on 100% RSBR was significantly higher than those of birds on control and other diets. This shows how the 100% RSBR diet was poorly utilized. This

confirmed the poor daily weight gains of birds fed 100% RSBR compared to birds fed on control and other diets. This is in agreement with the findings of Isikwenu *et al.* (2007) and (2008).

The selectivity in feed intake with relative wastage observed in the first two weeks of the starter phase with birds placed on 100% RSBR diet could be attributed to the high fibre content of the 100 % RSBRdiet which increases the bulkiness of the diet. This was also responsible for the wet litter often observed by birds on 100% RSBR diets. High fibrer diets have been observed (Church and Pond, 1988) to speed up the rate of passage of feed material through the simple stomach of monogastrics and that further more fibrous feeds may increase the output of nitrogenous material in the feaces. Atuahene (1986) attributed the cause of reduced voluntary feed intake by birds placed on 100% cotton seed cake to the high fibre content. The depressed feather development observed with birds on 100% RSBR diet during the starter phase was a reflection of the quality of RSBRdiet that was solely fed to the birds. Church and Pond (1988) revealed that the fibrous feeds might decrease the digestibility of crude protein and ether extract and increase the output of nitrogenous material into the faeces. The feed to gain ratio of birds on 100% RSBR diets was significantly higher than that for birds on other diets. This means that the feed was not efficiently utilized and there were no adequate nutrients (protein) for normal feather development. McDonald *et al*. (1998) identified delayed feathering as a symptom of sulphur containing amino acid (cysteine) deficiency that characterized proteins of microbial origin. According to McDonald *et al*. (1998), feathers were composed of a group of proteins known as □-keratins rich in sulphu amino acids (cysteine and methionine). Retarded growth and poor feathering wereobserved (Ofuya and Obilor, 1993) when fresh unfermented cassava peel was fed to broilers during the starter phase.

The carcass weight in relation to the live weights (dressing percentage) of birds on all diets except those on 100% RSBR diets were comparable to the standard (80%) for broilers at marketing (Barry,2006). These values of dressing percentages were similar to that reported by Okonkwo *et al* (1995) but higher than that reported previously (Ndams *et al*., 2009) and by Fanimo *et al.* (1998). The dressing percentage was observed to be affected in birds on 100% RSBR diets only. This could be attributed to the poor quality of the diet as reflected in the depressed daily gains of birds on 100% RSBR diet.

The breast percentages were affected by the dietary treatments in this study. The percentage breast of birds was observed to be depressed on RSBR supplemented diets compared to those on control. This findings is in agreement with that previously observed (Ndams *et al*., 2009) but is in conflict with that of Wylie *et al*., (2003) and Lemme and Petri (2002) who both observed increased breast percentage when wheat meal based diet were supplemented with amino acids. Birds on 25, 75 and 100% RSBRdiets had significantly lower percentage thigh compared to those on control and 50% RSBRdiets. This observation is in agreement with the findings of Lemme and Petri (2002) who reported a decrease in percentage thigh with higher dietary levels of lysine in wheat dietsas well as Ndams *et al*. (2009) who reported a decrease in percentage thigh with increased dietary supplementation of amino acid and enzymes in re-fermented brewers' dried grains (RBDG) diets.

All the PCV, Hb and TP values obtained in this study were within the normal range for birds as reported by Morton *et al*. (1994) and Aeangwanich *et al.,* (2004), and indicative of nutritional adequacy of dietary protein (Mohammed *et al.,* 2012). The packed cell volume (PCV) values ranged from 32.13 to 35.66 %, which fell within the normal range (22.0 – 35.0 %) for broiler

chickens (Aeangwanich *et al.,* 2004). According to Oyawoye and Ogunkunle (1998), a reduction in the concentration of PCV in the blood is indicative of the presence of a toxic factor which has deleterious effect on blood formation. There were no signs of toxic effects throughout the experimental period.

The results obtained for Hb followed to an extent a similar pattern with that of PCV indicating that the diets were nutritionally adequate to meet the proteins needs of the birds. Low Hb concentration according to Adeyemo and Longe (2007), apart from denoting anaemia but could also indicate animals on low protein intake, parasite infection or liver damage. Reduction in Hb count will lower effective oxygen transportation. The increase in Hb concentration observed in this study with RSBR supplementation of diets is in agreement with the reports of some authors who observed increments in Hb values on addition of green materials in broiler diets (Madubuike and Ekenyem, 2006; Nworgu *et al*., 2007). On the contrary, Olugbemi *et al*. (2010) observed non-significant increase in Hb values when 20 and 30% of Moringa oleifera leaf meal was introduced in cassava based broiler diets while Tewe (1991) also observed insignificantly lower Hb values when pigs were fed on 40% whole cassava meals.

Total protein was highest (3.6) in 50%RSBR diet and lowest (2.67) in 100% RSBR. This was probably a reflection of the protein quality in RSBR, as alluded to by Medugu *et al.*(2010). Similar results were obtained by Egyum (1970), Iyayi and Tewe, (1998) and Awosanya *et al.*(1999). It can be seen in this study that the total protein concentrations decreased as the dietary levels of RSBRincrease in the diets. This might explain partially the poor utilization of RSBR by the birds at relatively higher dietary inclusion levels (Agbabiaka *et al.,* 2013).

5.7.0 Performance of broiler chickens fed 75% urea and methionine-treated and fermented sorghum beer residue diets supplemented with specific multi-enzymes

In this study, birds group on enzymes supplemented diets showed uniform growth with good health, and less incidence of passing wet droppings compared to those group on un-supplemented diet. The excessive wetness of litter by birds on the 75%RSBR diet could be attributed to the high level of fibre content in the diet compared to that in the enzymes supplemented diets. This findings is in agreement with Biofeed Technology Inc. (Canada) producers of Fullzyme® who indicated its useful benefits on performance of broiler chicks to include greater uniformity, better health, improved litter and chick wellbeing. While the beneficial effects of Zymos-N® on broiler chickens were enumerated by Vamso PVT. Ltd (India) to include flock uniformity, better health and productivity, enhancement of tissue repair for better intestinal function and maintenance of health of liver, immune and other vital organs by improving tissue integrity. Ward and Beer (2012), linked the use of microbial protease to improved performances of birds during scourges of coccidiosis. The wet droppings observed with birds on 75%RSBRdiet is in agreement with the report of Osman and Elwarth (2007). Who affirmed that enzymatic growth promoters decreased wet litter syndrome and ammonia production in broiler houses and reduced the residence time of nutrients in the gastrointestinal tract by improving feed digestibility which gives less opportunity for growth of pathogenic bacteria.

Petterson (1987) reported that the use of high fibre diets increased the incidence of wet and sticky droppings in broilers. The higher feed intake, faster growth rate and better feed

conversion observed after 28days with birds fed +N and +Z diets compared to the control diet agrees with the findings of Garcia *et al*. (2003) and Lazaro *et al*. (2003).They observed significant improvement in the weight gain of birds fed barley, rye wheat and corn based diets supplemented with fungal enzymes preparation. Likewise Shakouri *et al*. (2009) found that the addition of enzymes (xylanase and □-glucanase) increased 28-day weight gain by 19.3% in broilers offered wheat-based diets but did not alter growth rates of birds offered sorghum-based diets. Myashkauskene *et al.* (1984) reported that use of an enzyme in broiler feed caused greater proteolytic activity in the stomach and duodenum that ultimately improved digestibility of crude protein.

The better feed conversion observed from birds on control compared to those on enzymes supplemented diets is in agreement with the findings of Khattak *et al*. (2006) who reported lower (better) feed conversion and not in agreement with the finding of Khan *et al*. (2006). While Khattak *et al*. (2006) observed better feed conversion by birds fed Rovabio® enzyme based diets compared to birds on the control diet, Khan *et al*. (2006) observed poor feed conversion by birds fed Hamecozyme® enzyme based diets compared to those on control.Noy *et al.* (2001)affirmed that digestibility lags early in life, indicating that young birds might benefit from supplemental enzymes. The faster weight gain of birds on +N and +Z enzymes supplemented diets over the control and 75%RSBR diets could be attributed to the availability of nutrients made from the increase digestibility of 75% RSBRby the microbial enzymes as observed by Ohta and Ishibashi, (2000).

The better feed conversion recorded from birds group on enzymes supplementation over the un-supplemented diet during this period of study (starter phase) conform to the findings of Hassanein (2011) who recorded significant improvement in feed conversion from birds on enzyme (Amecozyme) supplemented diets compared to other diets without enzymes. The results of feed cost per kg gain from birds fed the enzyme supplemented diets (+N, +Z and +F) were significantly lower than those from birds on control and un-supplemented diets. This indicated that enzyme-treated diets generated more profit than the control and un-supplemented diets and that enzymes supplementation is more feasible and economical to obtain maximum profitability from broiler production. The result indicated that all enzymes treated diets gave better economic efficiency than the control and the un-supplemented diets during the starter phase. This improvements in economic efficiency of all enzymes treated diets over the control and 75%RSBR diets were due to reduced total feed costs for the starter diets and the high body weight gain of such treatments. These results agree with Mateo and Carandang (2006) who found that the feed cost per kg gain was significantly low in corn-sorghum and sorghum based diets being 4.22 and 3.78% relative to corn based diet. Microbial protease have been reported (Ward and Beer, 2012) to help optimize feed cost per kg of meat in an increasingly difficult economic environment in Poultry Industry. Mikulski *et al*. (1999) also reported that enzyme supplementation decreased the relative cost of broiler feeds by 4 to 18% compared to that of non-enzyme supplemented feed.

The maximum mortality observed amongst birds group on 75%RSBR diet compared to those groups on enzymes treated diets in this present experiment could be attributed to the high fibre level of the 75%RSBR diet that caused increasing incidences of pasting vents and wet litter. The lower mortality recorded amongst birds on enzymes treated diets can be attributed to the

control of health pathogens, maintenance of liver health and other vital organs as well as enhancements of tissue repair for intestinal functions (Khan *et al*., 2006). This findings is in agreement with Strelec and Volk (1995) who reported that mortality rate was considerably low in enzymes treated groups. Samad *et al*. (2011) also reported significant decrease in mortality rate amongst enzyme-treatmented groups of birds compared to the untreated groups when probiotics were fed to broiler chickens in starter diets from 0-28days. Petterson (1987) reported that the use of high levels of fibre diets increased the incidence of sticky droppings in broilers.

Acamovic (2001) reported that enzymes increased the digestibility of feed ingredients and reduced the incidence of wet droppings which may result from the presence of fibrous polysaccharides in diets. Ward and Beer (2012) linked proteases with improved performance during times of coccidiosis challenges. The mitigation of sticking droppings in broiler chickens caused by sunflower-corn based diets by enzymes treatment was reported by Khan *et al*.(2006). Khan *et al*. (2006) observed change in microbial flora and better gut health when sunflower-corn based diets were supplemented with enzymes such as Rovabio and Hamecozyme-II. Morgan and Bedford (1995) reported the prevention of coccidiosis problems by using enzymes where birds fed a wheat-based diet with and without glucanase supplementation showed vastly different responses to coccidiosis challenge. While growth was depressed by 52-55% in control group, only 30.5% depression was observed in the enzyme group.

Observation at the end of the 56days trial, showed that birds fed the enzyme-supplemented diets ate and grew significantly fasterand tended to have better feed conversion ratio than those on75% RSBR diet. The better gains observed could be attributed to the nutrients made

available from the breakdown of supplemented diets by microbial enzymes (Nutriad, Zymos-N and Fullzyme) as observed by Ohta and Ishibashi (2000). The enzymes mixtures were claimed to breakdown the non-starch polysaccharides (NSP), reduce the viscosity of the gut content and improve the utilization of nutrients as reported by Khan *et al*. (2006). According to Myashkauskene *et al*. (1984), the use of enzyme (protease) in broiler feed caused greater proteolytic activity in the stomach and duodenum that ultimately improved digestibility of crude protein.

The lower body weight gain recorded among birds fed the un-supplemented diet (75% RSBR) may be attributed to the high fibre content of the diet (Farrel, 1994; Attia *et al*., 2003). Also, Makinde *et al*. (2013) had earlier reported that poorer body weight gain observed among birds fed high fibre diets could be as a result of the high fibre content of the diets. Addition of Natuzyme® enzyme generally improved fed utilization. Oladunjoye and Ojebiyi (2010) reported that broiler chickens can tolerate up to 20% rice bran in their diet at finishing phase when the diet was supplemented with Roxazyme G2G.

The better feed conversion exhibited by birds on enzymes treated diets signified the effectiveness of the enzymes supplemented diets to maintain adequate weight gain at the finisher phase. This present results agrees with the findings of Garcia *et al*. (2003) and Lazaro *et al*. (2003) who reported that fungal enzymes preparation significantly improved the weight gain of birds fed barley, rye and corn based diets. These findings were in agreement with Wu *et al*. (2003) and Ravindran *et al*. (2003). They observed improved weight gains and better feed

conversion when broilers were fed maize and wheat based diets supplemented with multi-enzyme (Allzyme).

The superior feed intake observed for birds on +N and +Z enzymes supplemented diets over the control and 75%RSBR diets could be as a result of the improved palatability of the enzymes supplemented diets. This is not in agreement with Ibrahim *et al*. (2012) who indicated that inclusion of the □-glucanase enzyme in sorghum-based diet significantly decreased total feed intake and significantly improved weight gain and feed conversion ratio of broilers. The lower feed cost/kg gain observed from birds on enzyme supplemented diets over the control and the 75%RSBR diets is in agreement with Khan *et al*. (2006), who indicated that enzyme supplementation was more feasible and economical to obtain maximum profitability from broiler production. Mikulski *et al*. (1999) also reported that enzyme supplementation decreased the relative cost of broiler feeds by 4 to 18% compared to that without enzyme supplementation.

The lower dressing percentage observed among birds fed on un-supplemented diet compared to those on diets with enzymes supplementation showed that enzymes treated 75%RSBRbased diets improved significantly the dressing percentage of birds. This is in agreement with Khan *et al*. (2006) who observed improvement in the dressing percentage of broilers fed enzyme supplemented sun flower-corn based diets compared to birds on control diet. The percentages of breast and thigh of birds on the un-supplemented diets were observed to be most significantly depressed compared to that of birds on other diets, while that of birds on +N and

+F were most superior to that of birds on the control. The depressed breast and thigh percentagesobserved with birds on 75%RSBR dietrevealed the superiority in quality of the control diet (100% GNC) which furnished adequate nutrients for breast and thigh developmentcompared to the 75%RSBR diet (25% GNC) that was higher in fibre. The superiority in breast % by birds on supplemented diet over those on 75%RSBR diet was an indication of the enzymes induced improvement in the feeding value of all 75%RSBR diets by the exogenous enzymes (+N, +Z, and +F). This finding is in agreement with Wylie *et al*. (2003) and Lemme and Petri (2002) who observed increased breast % when a wheat meal based diet were supplemented with amino acids, but is in disagreement with Lemme and Petri (2002) who also reported decreased percentage thigh with higher dietary levels of lysine in wheat based diets. The highest liver percentage numerically observed with birds on the un-supplemented (75%RSBR) diet compared to the other diets is in disagreement with Ndams *et al*. (2009), who observed significantly higher percentage liver for birds fed un-supplemented (30% RSRBDG) diet compared to those on diets supplemented with lysine, methionine and Allzyme (SSF) combination and control. The insignificant difference in percentage liver observed with birds on +N, +Z and +F and the control diets is in agreement with Ndams *et al*. (2009) who observed similar percentage liver for birds on the control and the supplemented lysine, methionine and enzyme combination. This could be attributed to the digestibility of the enzymes supplemented deits as compared to the control diets. This is not in agreement with Fanimo *et al*.(1998) who observed an increase in liver weight % with increased level of amino acids and contrary to the report of Brenes *et al*. (1993) and Atteh (2004) who observed a decreased liver percentage. The higher the fibrous nature of the diet, the higher the activities of the liver and the higher the expected size of liver and vice-versa (Ndams, *et al*., 2009). The higher percentage size of the liver

of birds on 75%RSBR diet was an indication of the low quality of the diet compared to other diets.

The percentage gizzard in this study was significantly higher for birds on the 75%RSBR diet compared to other diets. This agrees with the findings of Khan *et al*. (2006) who reported that enzymes treatment reduced significantly, the size of proventriculus and gizzard. Similarly Viveros et *al*. (1993), reported that addition of enzyme to barley-based diets also reduced the relative weight of upper tract (mainly proventriculus and gizzard). In this study, the percentage heart, abdominal and intestinal lengths were all affected by the different treatments. This is in agreement with Ndams *et al*. (2009) who observed differences in percentage heart, gizzard and abdominal fat as a result of 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 significantly higher percentage of heart and intestinal length observed with birds on 75%RSBR diet compared to that of birds on other diets could be attributed to the high fibre in the un-supplemented diet compared to the control and enzymes supplemented diets whose enzymes activities has reduced their fibre content. This is also in agreement with the findings of Okonkwo *et al*. (1995) who observed that high fibre diets induced significant increase in the weight and size of the various sections of the digestive system of birds. The high fibre content of the 75%RSBR diet caused the gizzard and heart to adjust in size to handle the higher fibre content of the diet.

The lower intestinal length showed by birds on enzymes supplemented diets compared to that of birds on un-supplemented diets is in agreement with the findings of Marquardt *et al*.(1996) who reported that enzyme supplementation reduced the lengths and sections of various parts of the gastro-intestinal tract. While Ndams*et al.* (2009) on the contrary did not observe any effect on the intestinal length of birds (broilers) when re-fermented brewers' dried grains was fed to broilers. Dogari (1985) also observed a non-significant effect on intestinal length when maize was replaced at different levels by sorghum beer residue in broiler diets. The higher significant ($P<0.05$) abdominal fat of birds on the 75%RSBR diet compared to the control and enzymes supplemented diets is contrary to the findings of Fanimo *et al*. (1998). The researchers observed an increase in abdominal body fat with lysine plus methionine supplementation of broiler diets.

CHAPTERSIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of the Major Findings

6.1.1 Determination of the effect on nutrient composition of sorghum beer residue passed through a second fermentation process

i. Fermented SBR samples on 10:4 substrate to inoculum concentration was Significantly ($P<0.05$) higher in crude protein compared to control and samples on 10:1, 10:2 and 10:3 substrate to inoculum concentrations.

ii. The CP content of samples on 1.5% urea treatment was similar to that of samples on 1.0% urea treatment but significantly ($P<0.05$) higher than those on the control, 0.5 and 2.0% urea treatment.

iii. The crude Protein content of all samples on 0.29, 0.33, 0.37 and 0.41% methionine treatment did not differ significantly ($P>0.05$) but were significantly higher ($P<0.05$) than the control.

iv. The increase in CP content was observed to be significantly ($P<0.05$) highest (38.19%) among samples on urea plus methionine ratio of 0.37 to 1.5% (N_3S_3).

v. Amino acid profiles (g/100g protein) of SBR and all fermented SBR samples revealed the availability of all essential amino acids that are required in poultry nutrition with the exception of tryptophan.

6.1.2 Effect of feeding urea plus methionine supplemented and fermented sorghum beer residue on the performance and carcass characteristic of broiler chickens

i. Daily feed intake of birds on 50 and 75% RSBR were similar but significantly ($P<0.05$) higher than those on control, 25 and 100% RSBR during the starter phase, but during the finisher period, the daily feed intake of birds on the control and 25% RSBR diets were similar ($P>0.05$) and significantly ($P<0.05$) lower than those of birds on other diets.

ii. Daily weight gain of birds on 25 and 50% RSBR were similar ($P>0.05$) but significantly ($P<0.05$) better than those on control, 75 and 100% RSBR diets during the starter phase.The final and daily weight gains of birds fed the control and up to 50% RSBR during the finisher period were similar but significantly ($P<0.05$) better than those fed 75 and 100% RSBR diets.

iii. The feed to gain ratio of birds on 100% RSBR diet was significantly ($P<0.05$) higher than those on the control, 25, 50 and 75% RSBR diets at both the starter and finisher phases.

iv. During the starter and finisher phases, mortality was significantly ($P<0.05$) higher in birds on control compared to those on other diets.

v. The dressing percentage among birds on 50% RSBR diet was highest significantly ($P<0.05$) compared to those of birds on other diets. The breast weight and thigh percentages for birds on the control was observed to be highest significantly ($P<0.05$) while that for birds on 100% RSBR diet to be the lowest significantly ($P<0.05$).

6.1.3 Performance of broiler chickens fed 75% urea and methionine treated and fermented sorghum beer residue diets supplemented with specific multi-enzymes

i. During the starter phase, the final and daily weight gains of birds on Nutriad enzyme and those on diet supplemented with Zymos-N were observed to be similar ($P>0.05$) but significantly ($P<0.05$) higherwhen compared to those of birds fed on the control, 75%RSBR and Fullzyme diets. The final and daily weight gains of birds on diet Nutriad enzyme was significantly ($P<0.05$) higher than those on other treatments during the finisher phase.

ii. The feed to gain ratio of birds on control diet during the starter phase was significantly the highest among birds on all treatments while that for birds on 75%RSBR diet showed the lowest significantly ($P<0.05$).

iii. The feed to gain ratio of birds on +N diet was better ($P<0.05$) compared to those on control and other diets during the finisher phase.

iv. The dressing and thigh weight percentages for birds on +N diet was significantly ($P<0.05$) higher than those of birds on control and other diets.

6.2 Conclusion

From the studies, the following conclusions were arrived at;

i. 4-day fermentation of SBR samples on urea plus sulfur treatment ratio of 1.5: 0.39 resulted in the highest % CP(38.19%).

ii. The inclusion of RSBR in broiler diets at 50% levels at both starter and finisher phases resulted in higher final and daily weight gains.

iii. The use of 50% RSBR in the diets of broilers during the starter and finisher phases resulted in lower cost of feed per kg gain (~~N~~).

iv. Supplementation of 75% RSBR broiler starter and finisher diets with Nutriad enzymes resulted in superior weight gains.

v. Supplementing 75% RSBR diets with Nutriad and Zymos-N enzymes resulted in higher feed intake during the starter and finisher phases.

vi. Birds on all enzymes (Nutriad, Zymos-N and Fullzyme) supplemented diets exhibited superior feed conversion efficiency over those on un-supplemented (75% RSBR) diet at both starter and finisher period.

6.3 Recommendations of the study

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

i. Four (4) day commercial fermentation of SBR with rumen liquor at concentration of 10: 1 with the supplementation of urea plus methionine ratio of 1.5:0.37 for the enhancement of its protein biomass for broiler nutrition.

ii. The use of 50% RSBR in the diets of broilers as a means of improving performance and reducing pressure on conventional protein sources like soya bean and GNC.

iii. Adequate supplementation of 75% RSBR with Nutriad, Zymos-N and Fullzyme enzymes for use in broiler rations as a way of achieving better performance.

6.3.1 Recommendation for further studies

Further studies into the replacement of major sources of protein in the diet of broiler chickens with 100% urea andmethionine treated and fermented sorghum beer residue (100%RSBR) with multi-enzymes supplementation is recommended.

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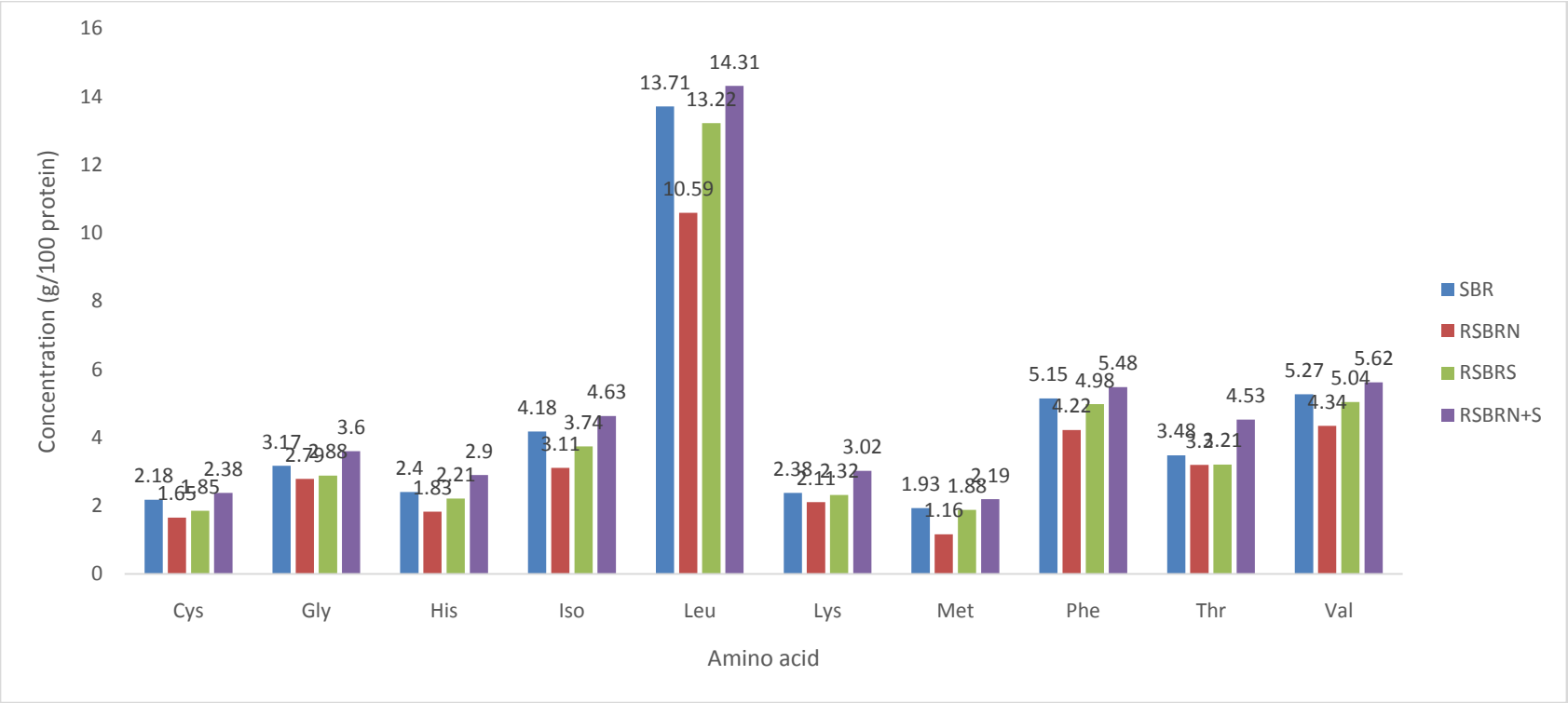
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LIST OF APPENDICES

Appendix 1. Comparison of the amino acid profiles of SBR, RSBRN, RSBRS andRSBRN+S



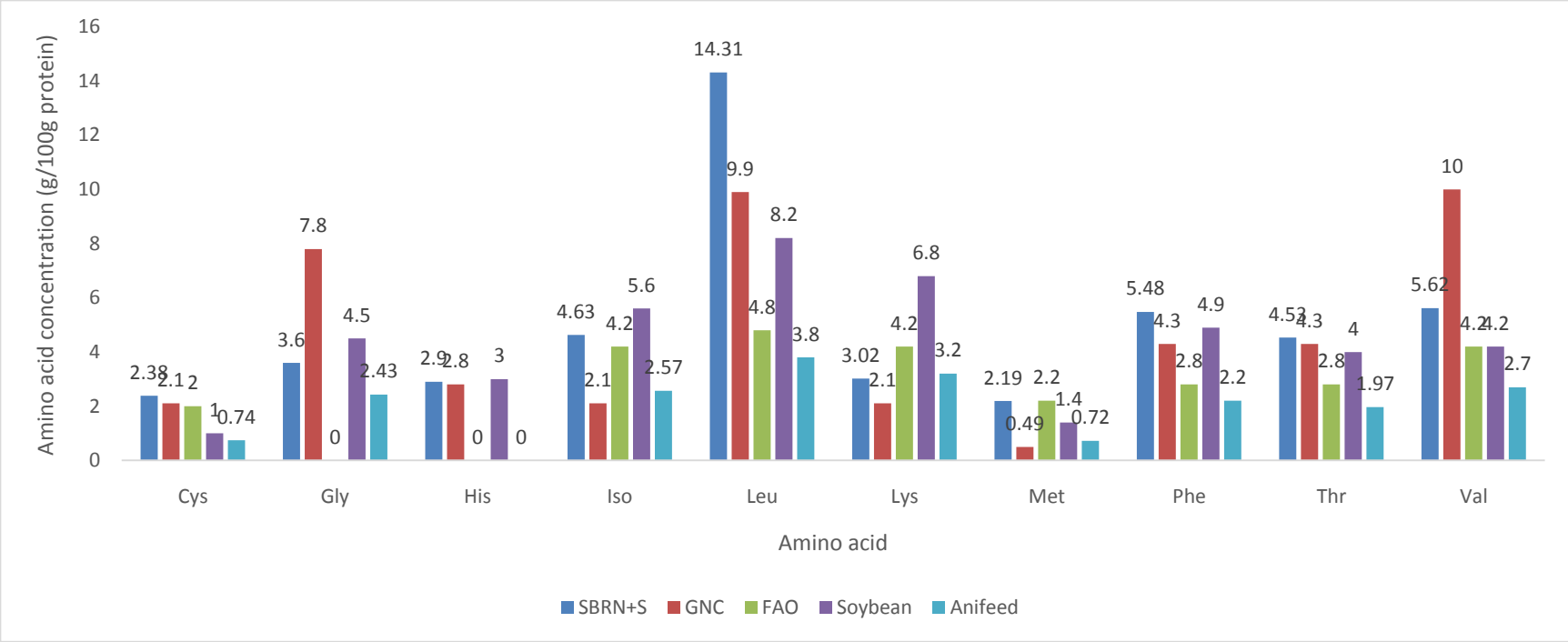
SBR = Sorghum beer residue

RSBRN = Refermented SBR with urea supplementation

RSBRS = Refermented SBR with methionine supplementation

RSBRN+S = Refermented SBR with urea plus methionine supplementation

Appendix 2. Comparison of the amino acid profile (g/100g protein) of the protein biomass produced (RSBR) with that of other protein standards



SBRN+S = Fermented sorghum beer residue with urea plus methionine supplementation

GNC = Groundnut cake

FAO = Food and Agricultural Organization feed standard

Anifeed = Animal feed standard

Appendix 3: Filtration of rumen content through mosquito net (pore size of 2.78mm^2) for inoculation of sorghum beer residue



Appendix 4: Sixteen (16) samples of sorghum beer residue treated with different urea to methionine ratios (Table 3.4) and fermentedfor 4 days in empty jam containers with covers.



Appendix 5: Five feet (5ft) deep pit used for bulk fermentation of sorghum beer residue



Appendix 6: Compaction of urea plus methionine treated sorghum beer residue in the 5-feet deep pit for anaerobic fermentation



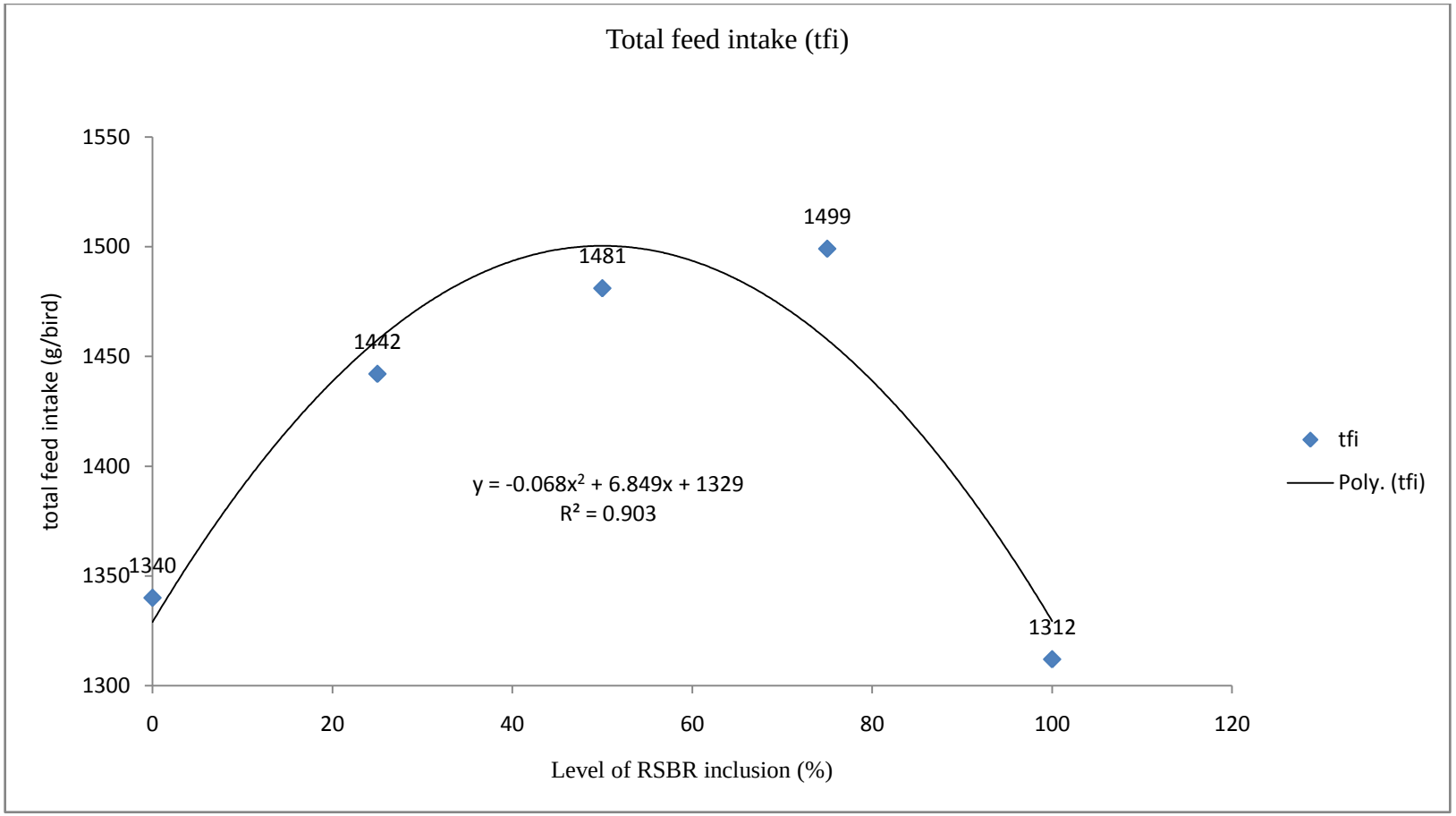
Appendix 7:Urea plus methionine treated sorghum beer residue after 4 days fermentation



Appendix 8: Processed refermented sorghum beer residue (RSBR) ready for use as feed ingredient

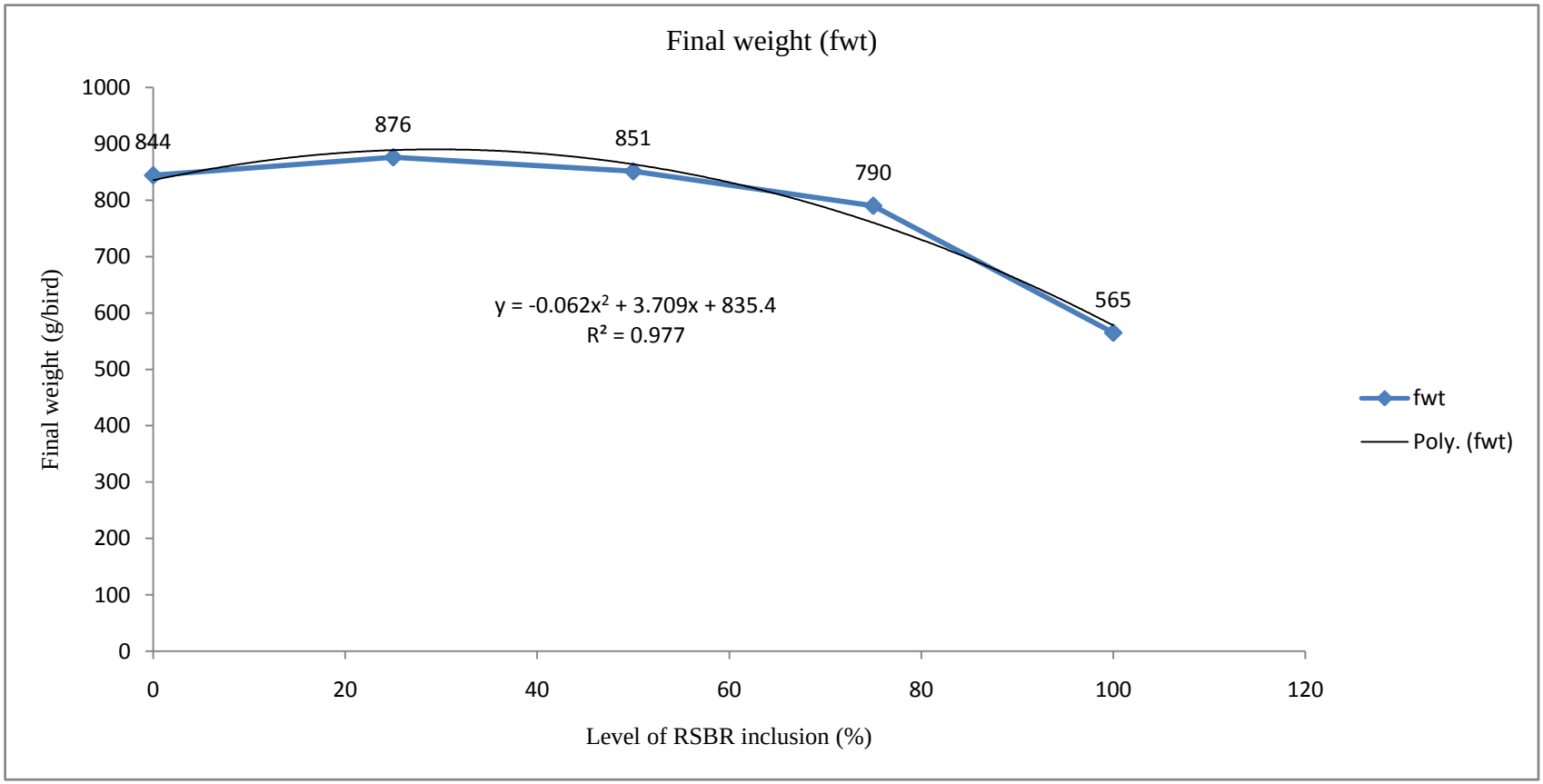


Appendix 9: Trend line of total feed intake of birds fed RSBR diets (0-4 weeks).



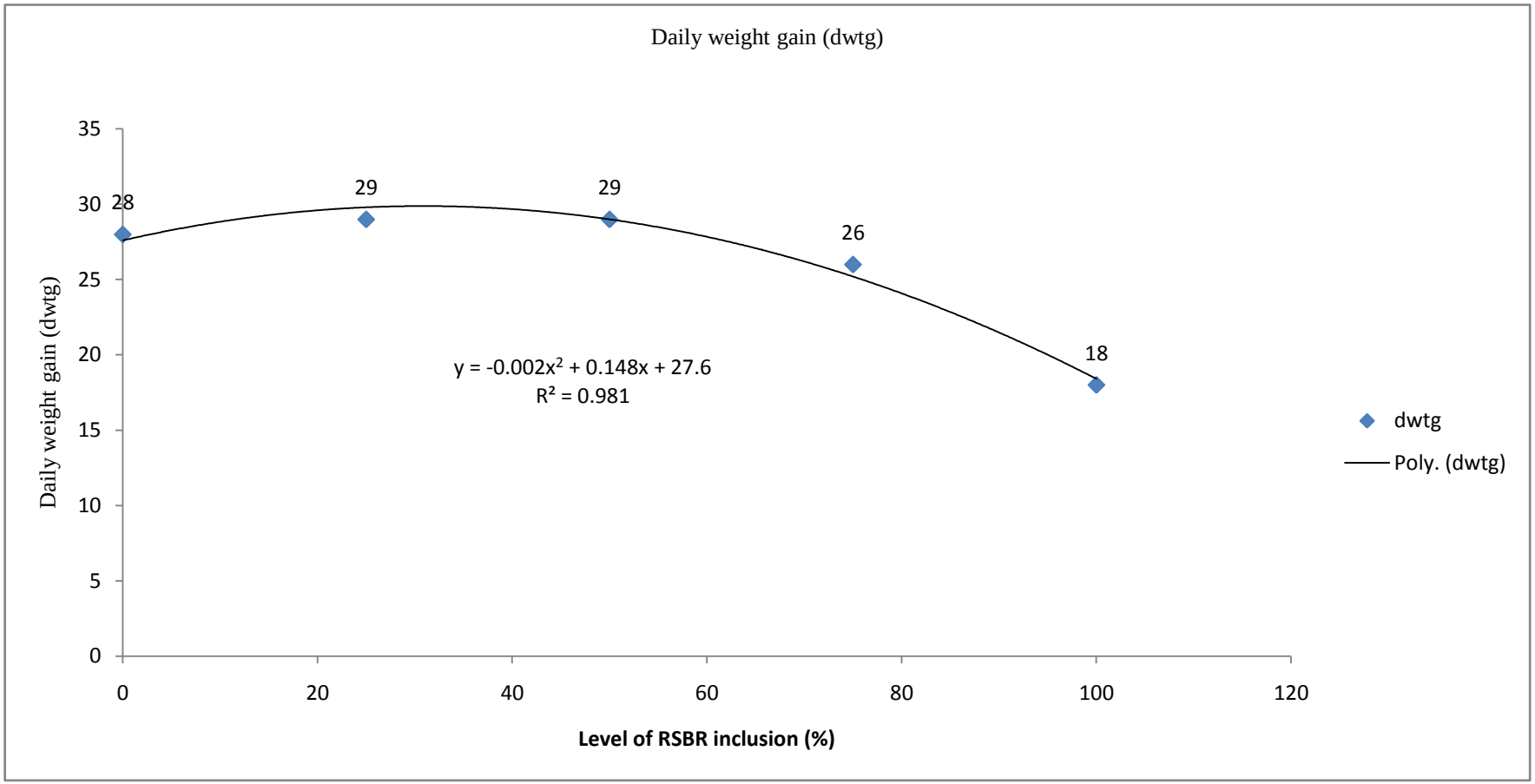
RSBR = refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

Appendix 10: Trend line of final weight of birds fed RSBR diets (0-4 weeks).



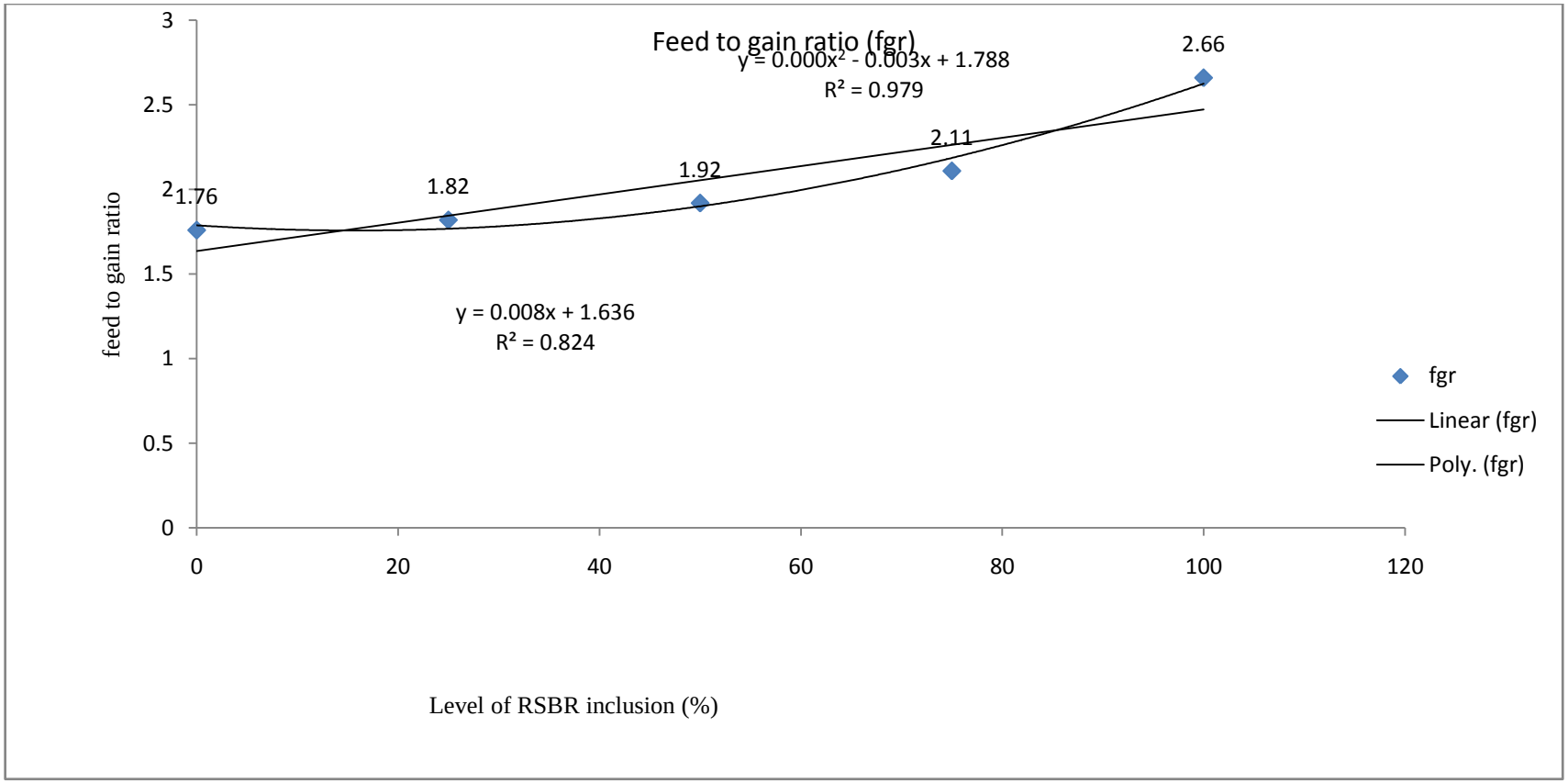
RSBR = refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

Appendix 11: Trend line of daily weight gain of birds fed RSBR diets (0-4 weeks).



RSBR = Refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

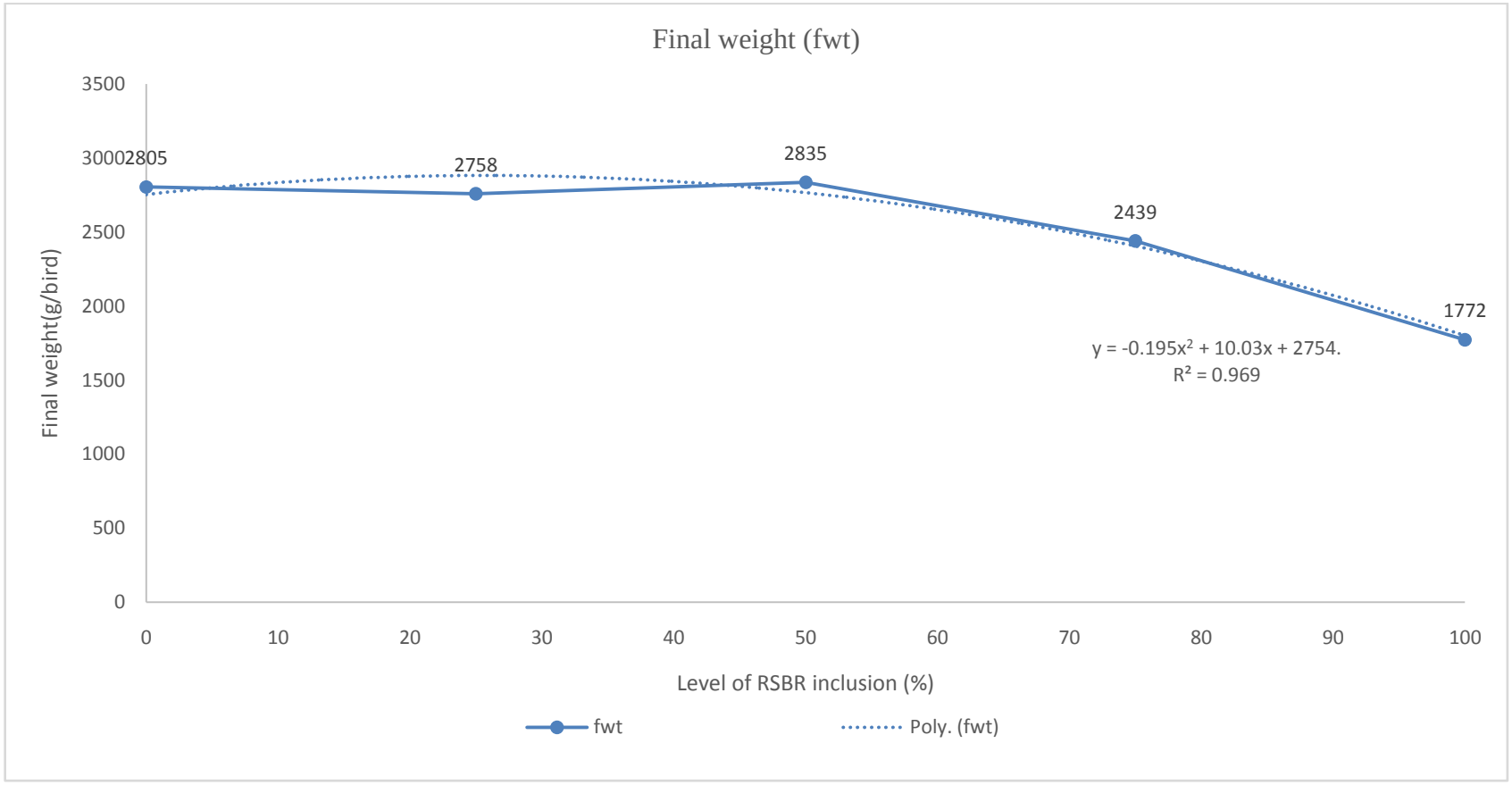
Appendix 12: Trend line of feed to gain ratio of birds fed RSBR diets (0-4 weeks)



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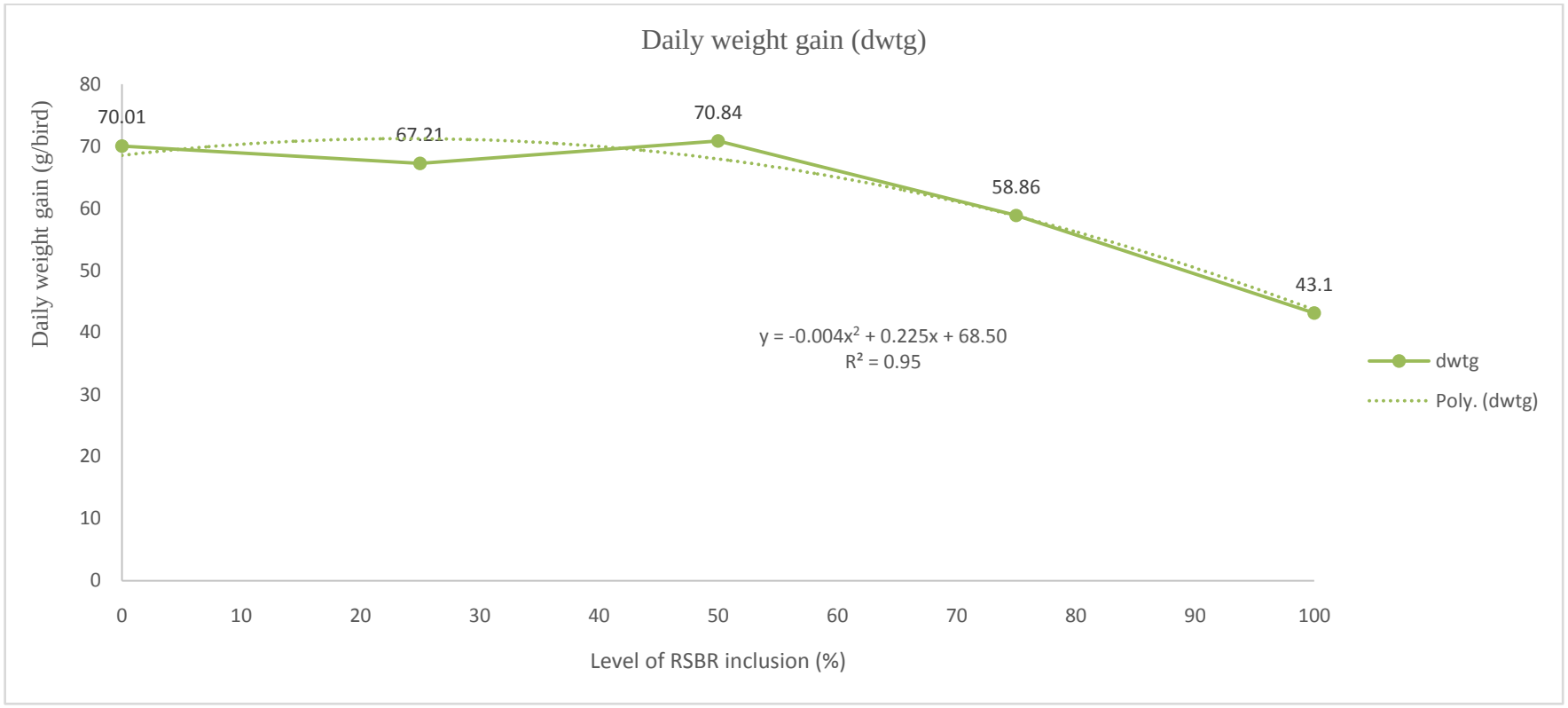
RSBR = Refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

Appendix 13: Trend line of final weight of birds fed RSBR diets (4-8 weeks).



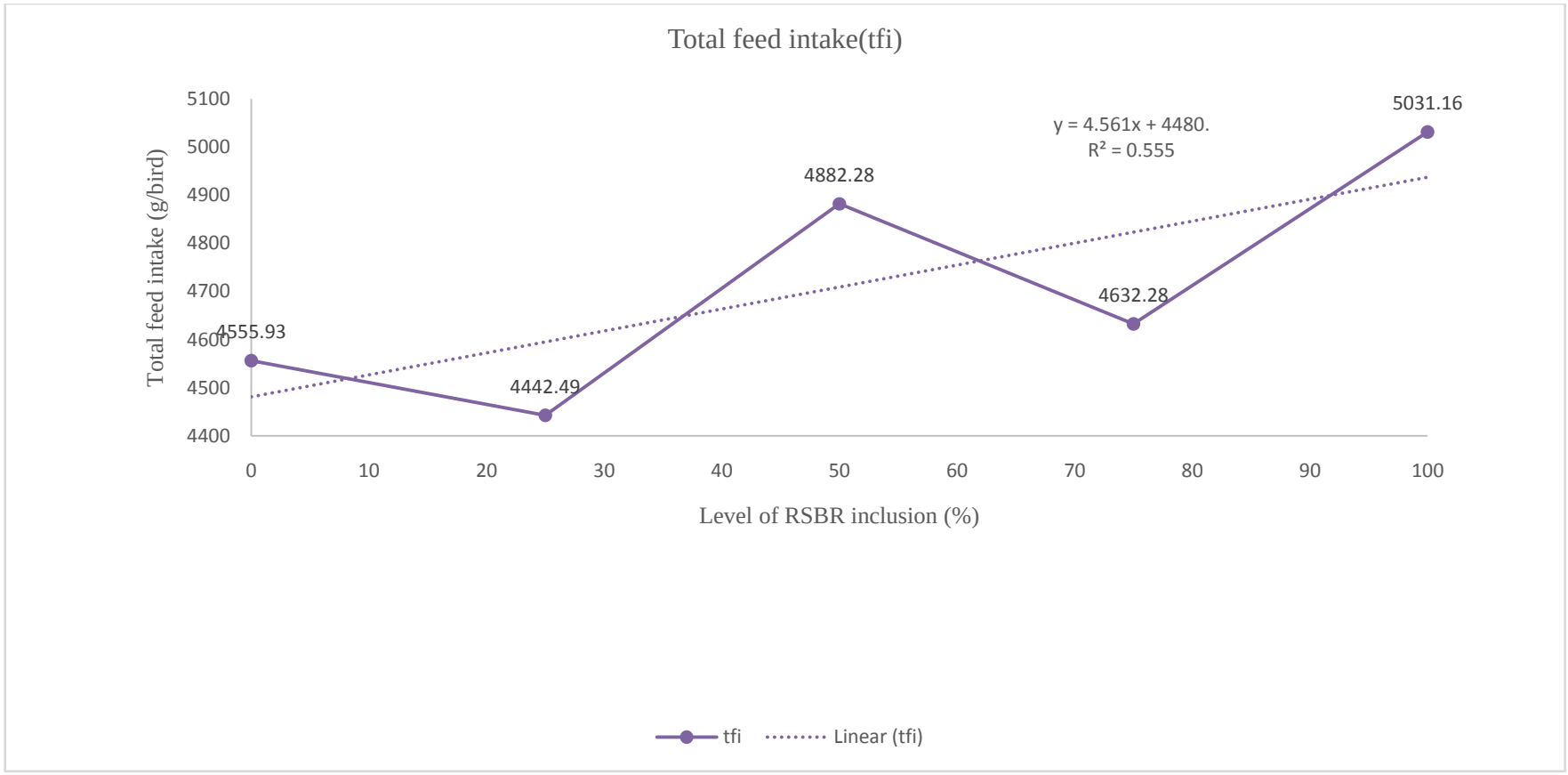
RSBR = Refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

Appendix 14: Trend line of daily weight gain of birds fed RSBR diets (4-8 weeks).



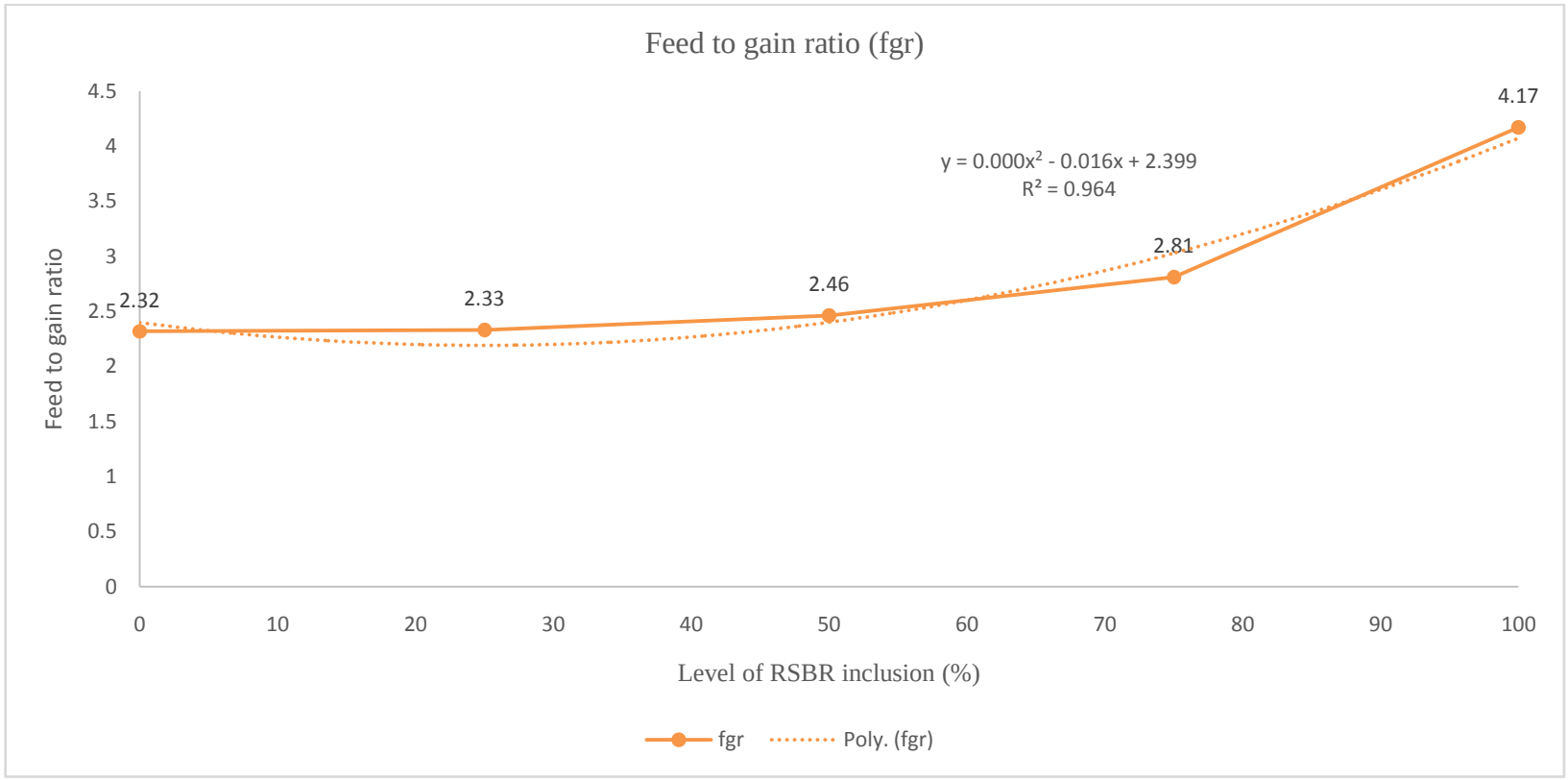
RSBR = Refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

Appendix 15: Trend line of total feed intake of birds fed RSBR diets (4-8 weeks).



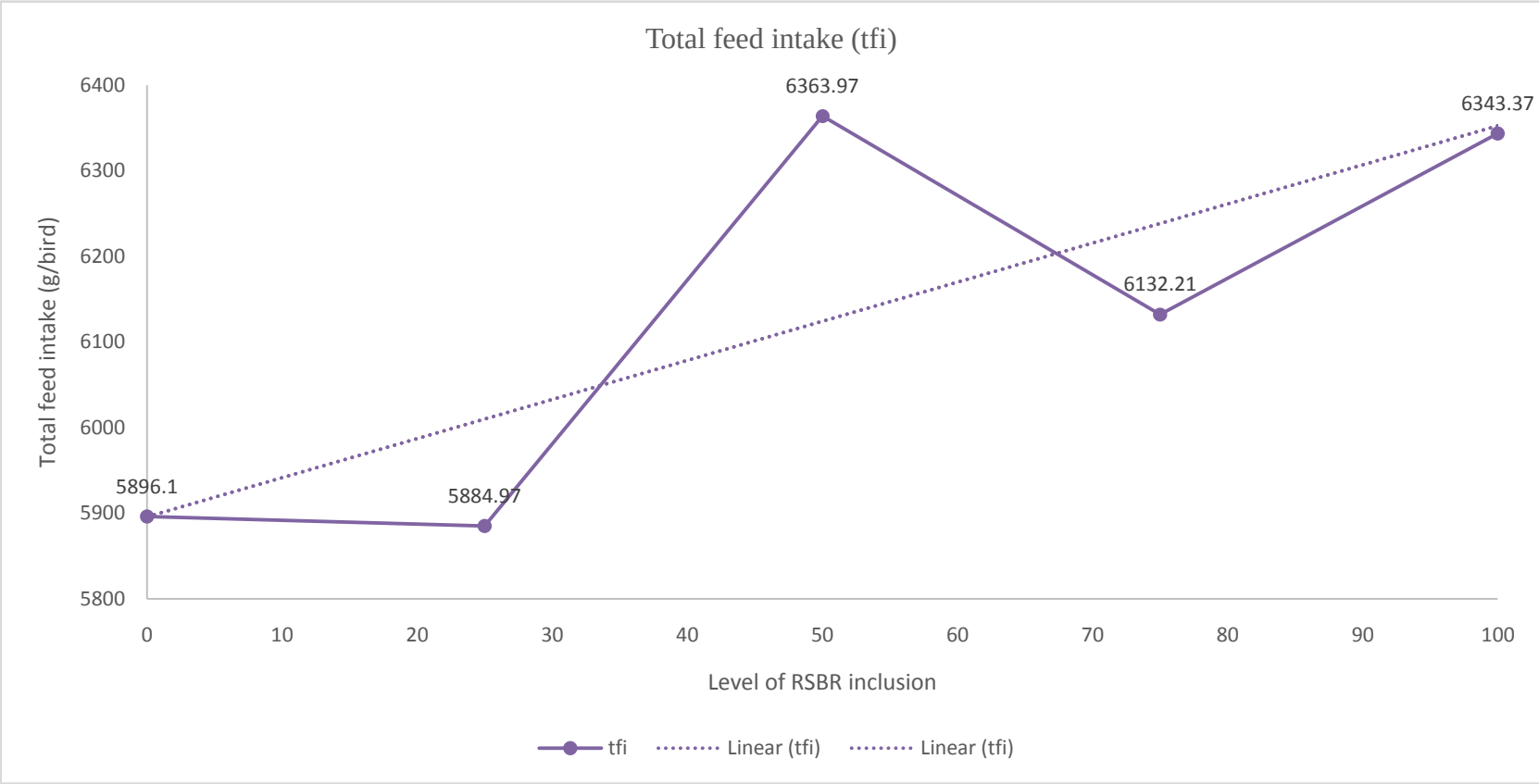
RSBR = Refermented SBR supplemented with urea plus sulfur.

Appendix 16: Trend line of feed to gain ratio of birds fed RSBR diets (4-8 weeks).

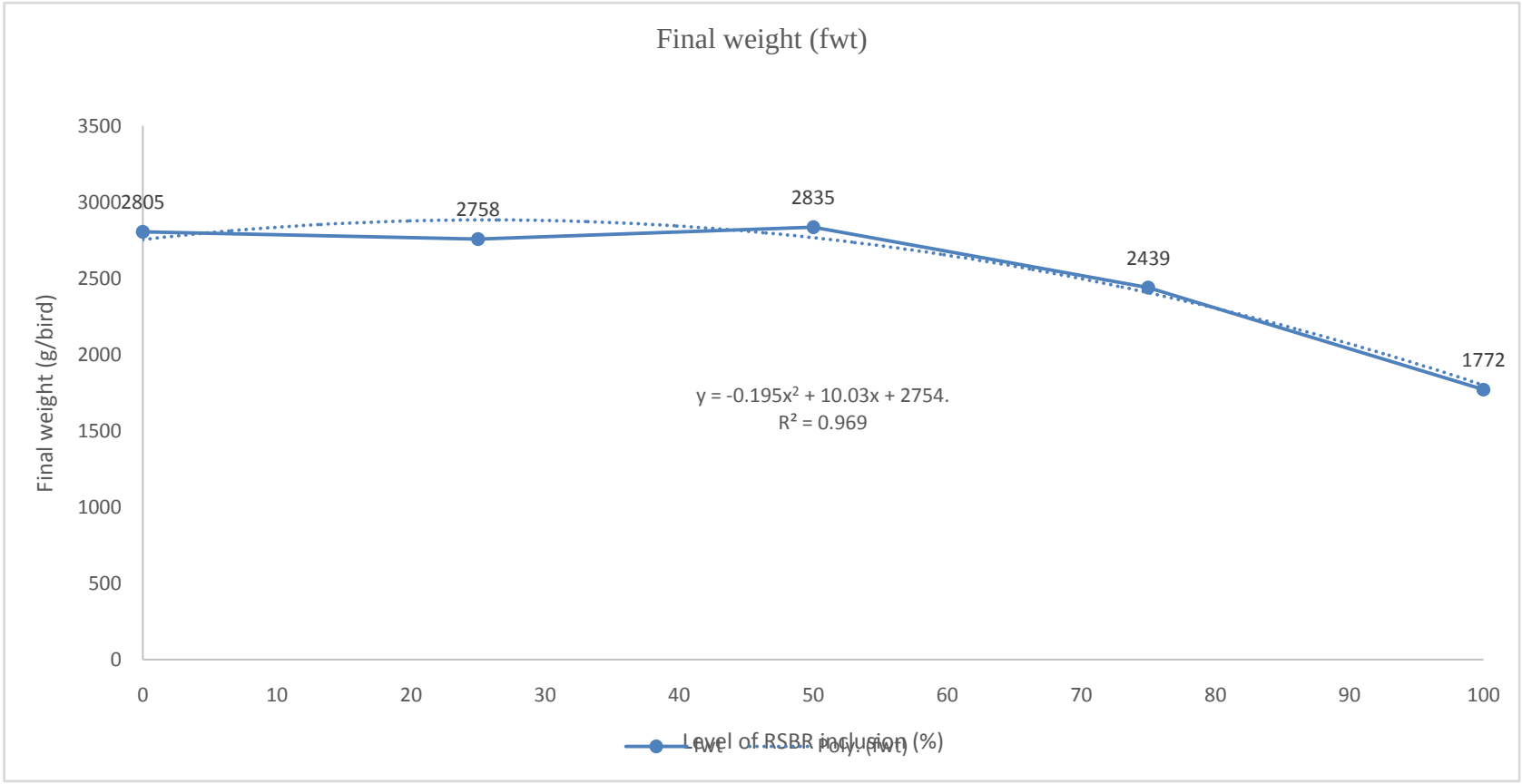


RSBR = Refermented SBR supplemented with urea plus sulfur.
Poly = Polynomial

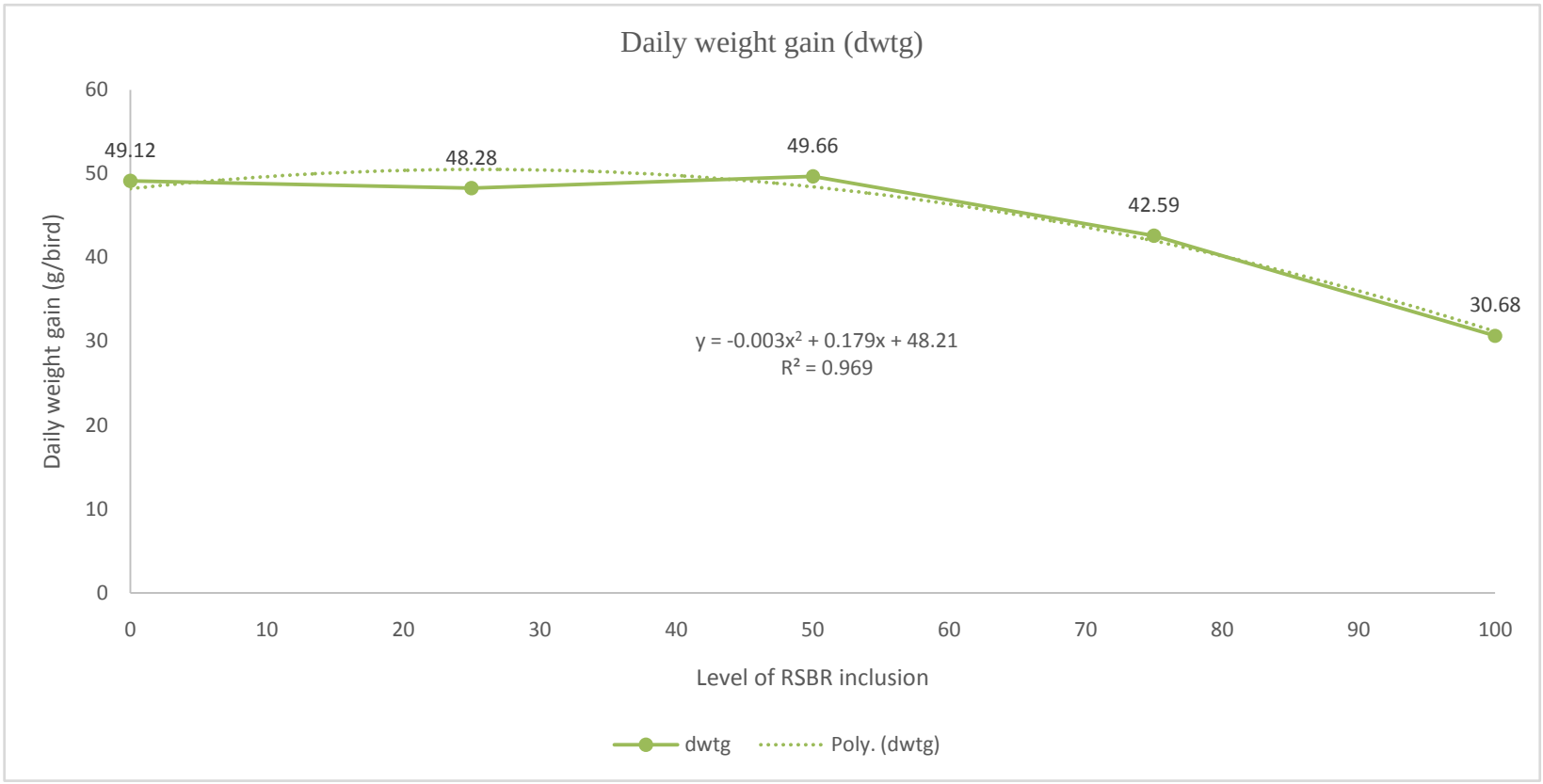
Appendix 17: Trend line of total feed intake of birds fed RSBR diets (0-8 weeks).



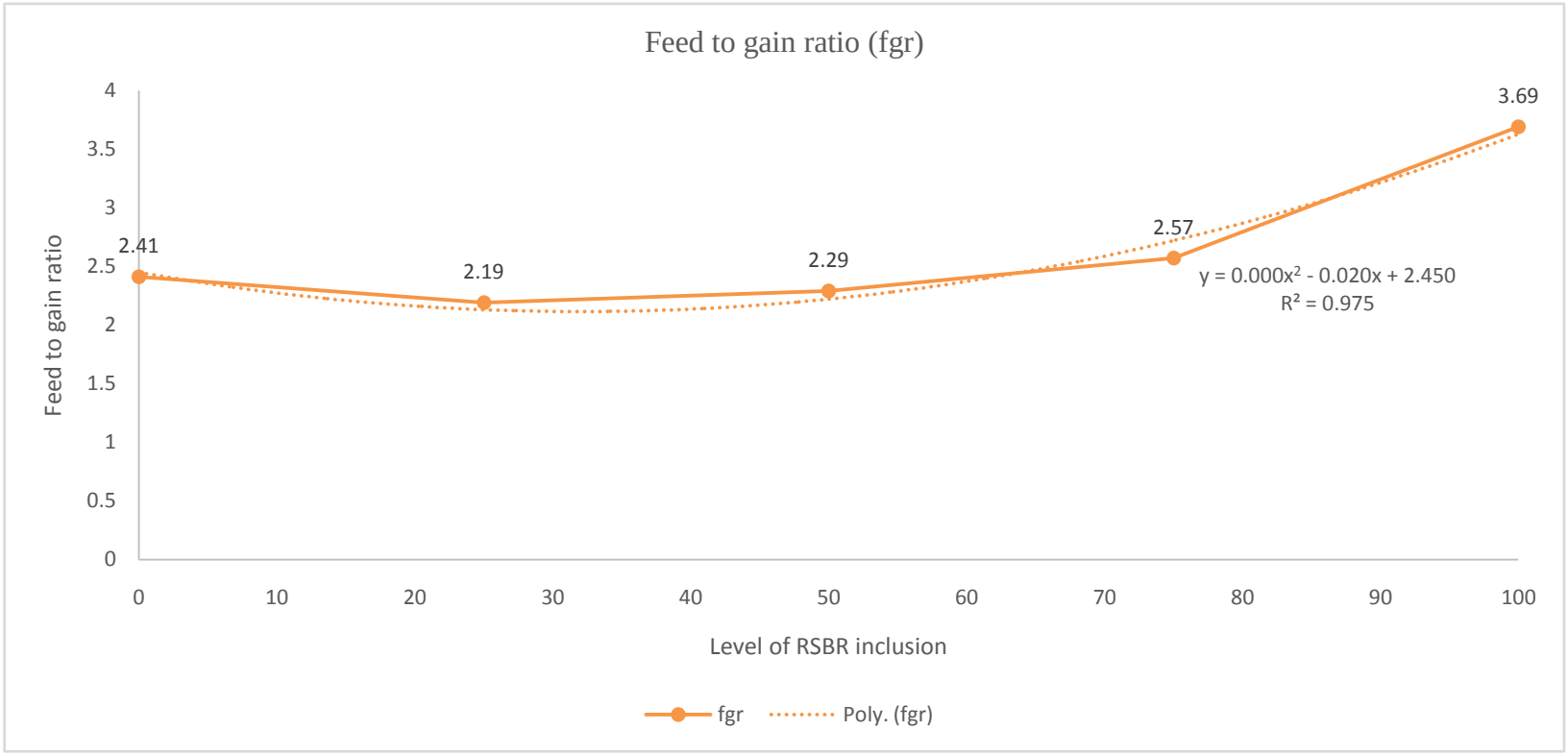
Appendix 18: Trend line of final weight of birds fed RSBR diets (0-8 weeks).


Final weight (fwt)
Final weight (g/bird)
3500
3000
2500
2000
1500
1000
500
0
2805
2758
2835
2439
1772
y = -0.195x² + 10.03x + 2754.
R² = 0.969
0
10
20
30
40
50
60
70
80
90
100
Level of RSBR inclusion (%)
fwt
Poly. (fwt)

Appendix 19: Trend line of daily weight gain of birds fed RSBR diets (0-8 weeks).



Appendix 20: Trend line of feed to gain ratio of birds fed RSBR diets (0-8 weeks).


Feed to gain ratio (fgr)
Feed to gain ratio
Level of RSBR inclusion
2.41
2.19
2.29
2.57
3.69
y = 0.000x² - 0.020x + 2.450
R² = 0.975
fgr
Poly. (fgr)