

**GROWTH PERFORMANCE, GUT MICROBIAL PROFILE AND CARCASS  
CHARACTERISTICS OF GROWING PIGS FED DIETS CONTAINING THREE  
DIFFERING (RE3, RE3 PLUS and P3) DFM PRODUCTS**

**BY**

**SOLOMON OSARUMWENSE OKUNGBOWA B. Sc. (HONS)**

**A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE,  
KWAME NKRUMAH UNIVERSITY OF SCIENCE AND  
TECHNOLOGY  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE**

**OF**

**MASTER OF SCIENCE  
(ANIMAL NUTRITION)**

**FACULTY OF AGRICULTURE**

**COLLEGE OF AGRICULTURE AND NATURAL RESOURCES**

**FEBRUARY, 2014.**

## DECLARATION

I, Solomon Osarumwense Okungbowa, declare that this thesis is my own work towards the award of a degree in this university and it contains no material which has been published by another person or being submitted for the award of any other degree of the university or elsewhere. However, works of other researchers used as sources of information have been acknowledged by reference to the authors.

# KNUST

Solomon Osarumwense Okungbowa .....  
(PG5303711) Signature Date  
(Candidate's Name and ID)

Certified by:

Prof. D. B. Okai .....  
(Co-project Supervisor) Signature Date

Dr. M. Boateng .....  
(Co-project Supervisor) Signature Date

Dr. (Mrs.) V. Attoh-Kotoku .....  
(Head of Department) Signature Date

## ABSTRACT

A twenty-two week feeding trial was conducted at the Livestock Section of the Department of Animal Science, Faculty of Agriculture, KNUST, Kumasi. This study sought to investigate the effects of three (3) differing Direct-fed microbial (DFM) products on growth performance, gut microbial profile and carcass characteristics of growing pigs. Twenty Large White pigs (12 females and 8 males) with an average initial weight of 9.4 kg were allocated to four dietary treatments labeled: T1 (Control), T2 (1ml RE3<sup>TM</sup> per kg feed), T3 (1ml RE3 PLUS per kg feed) and T4 (1ml RE3<sup>TM</sup> + 0.5ml P3 per kg feed) in a Completely Randomized Design (CRD) with 5 replicates per treatment. All the pigs were given access to feed and water *ad libitum*. There were 2 phases in this experiment. A starter phase where pigs were offered a 23% CP diet until they attained body weight of  $20 \pm 0.5$  kg and a grower-finisher phase where pigs were fed an 18% CP diet until they attained a body weight of  $70 \pm 0.5$  kg. Weighing was done weekly. Faecal samples were taken from all the pigs during the course of the experiment. After any individual pig attained a targeted bodyweight of  $70 \pm 0.5$  kg, it was slaughtered. Blood samples were then taken and fresh carcass parameters were taken. Carcasses were chilled in a cold room (4°C) for 24 hours after which chilled carcass parameters were taken. It was therefore concluded that the addition of the differing DFM products did not seem to influence growth performance, gut microbial composition and carcass characteristics of growing pigs.

# KNUST



# TABLE OF CONTENT

	PAGE
TITLE PAGE	i
DECLARATION	ii
ABSTRACT	iii
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF PLATES	x
LIST OF ABBREVIATIONS	xi
DEDICATION	xiii
ACKNOWLEDGEMENTS	xiv
<b>CHAPTER 1</b>	
<b>1.0 INTRODUCTION</b>	<b>1</b>
<b>CHAPTER 2</b>	<b>4</b>
<b>2.0 LITERATURE REVIEW</b>	<b>4</b>
2.1 Feed Additives	4
2.1.1. Benefits of Feed Additives	4
2.1.2. Types of Feed Additives	5
2.2. Antibiotics as Additives in Animal Feed	7
2.2.1. Mode of Action of Antibiotics	8
2.2.2. Growth Promotion Effects of Antibiotics	10

2.2.3.	Antibiotic Resistance	11
2.2.4.	Forms of Resistance	12
2.3.	Alternatives to Antibiotic in Animal Growth Promotion	14
2.3.1.	Acidifiers or Organic Acids	15
2.3.2.	Dietary or Exogenous Enzymes	16
2.3.3.	Clay Adsorbents	17
2.3.4.	Phytobiotics	18
2.3.5.	Prebiotics	19
2.3.6.	Direct-Fed Microbial (DFM) or Probiotics	20
2.3.6.1.	Microorganisms Used in DFM	20
2.3.6.1.1.	Bacterial DFM	22
a.	<i>Paenibacillus polymyxa</i>	23
2.3.6.1.2.	Fungal DFM	24
2.3.6.2.	Mechanism of Action of DFM	25
2.3.6.3.	Effects of DFM	26
2.3.7.	RE3™ as a DFM Product	29
2.4.	Summary of Literature Reviewed	31
<b>CHAPTER 3</b>		<b>33</b>
<b>3.0</b>	<b>MATERIALS AND METHODS</b>	<b>33</b>
3.1.	Site, Duration and Phases of the Experiment	33
3.2.	Animals and Design of the Experiment	33

3.3.	Housing	34
3.4.	Feeds and Feeding Regimes	34
3.5.	Health	35
3.6.	Management	35
3.7.	Parameters Measured	35
3.7.1.	Feed Intake	35
3.7.2.	Live Weight Changes and Weight Gains	35
3.7.3.	Feed Conversion Ratio (FCR)	36
3.7.4.	Feed Cost and Economy of Gain	36
3.7.5.	Carcass Evaluation	36
3.8.	Faecal Microbial Analysis	38
3.9.	Statistical Analysis	38
<b>CHAPTER 4</b>		
<b>4.0</b>	<b>RESULTS AND DISCUSSION</b>	<b>39</b>
4.1.	Health	39
4.2.	Growth Performance	40
4.2.1.	Starter Phase	40
4.2.2.	Grower-Finisher Phase	42
4.2.3.	Overall Performance (Starter-Finisher)	43
4.3.	Gut Microbial Count	45
4.4.	Carcass Characteristics	46

## CHAPTER 5

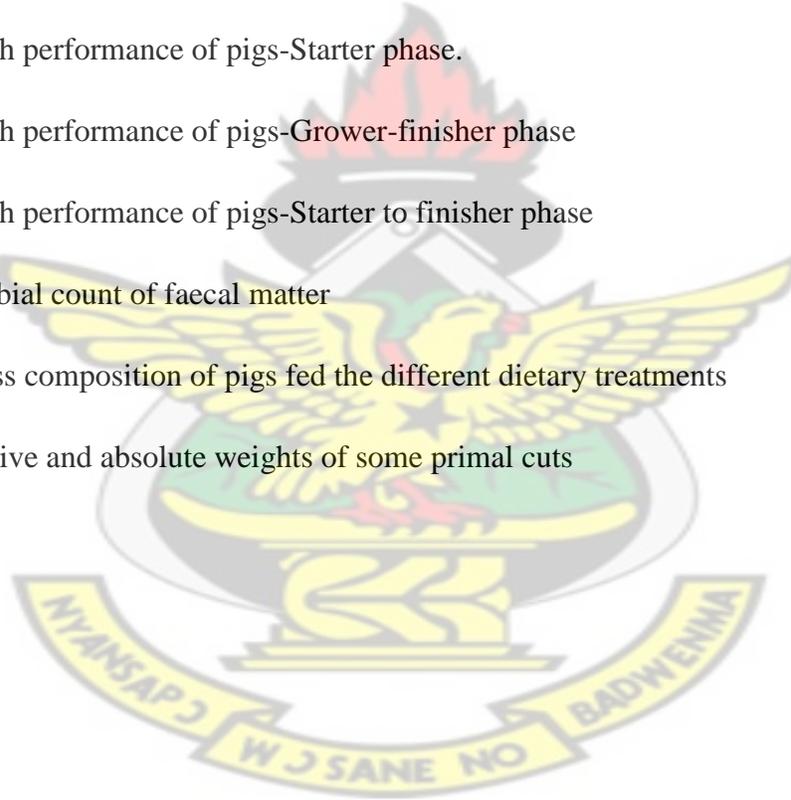
5.0	CONCLUSIONS AND RECOMMENDATIONS	51
6.0	REFERENCES	52
7.0	APPENDIX	66

# KNUST



## LIST OF TABLES

TABLE		PAGE
1	Some antibiotics, their effects and their mechanism of action	10
2	FDA and AAFCO Approved Microorganisms for use in DFM products	21
3	Composition of RE3™	30
4	Percentage composition of the experimental diets	34
5	Growth performance of pigs-Starter phase.	40
6	Growth performance of pigs-Grower-finisher phase	42
7	Growth performance of pigs-Starter to finisher phase	44
8	Microbial count of faecal matter	46
9	Carcass composition of pigs fed the different dietary treatments	47
10	Relative and absolute weights of some primal cuts	48



## LIST OF PLATES

PLATE		PAGE
1	Diagrammatic representation of the 3 forms of horizontal gene transfer	14

# KNUST



## LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
AAFCO	Association of American Feed Control Officials
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
ADWG	Average Daily Weight Gain
AGP	Antibiotic Growth Promoters
ANOVA	Analysis of Variance
BEST	Basic Environmental Systems and Technology
CP	Crude Protein
CRD	Completely Randomized Design
DFM	Direct - Fed Microbials
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
LSD	Least Significant Differences
NOAH	National Office of Animal Health
PCA	Plate Count Agar
UK	United Kingdom
USA	United States of America

VFA	Volatile Fatty Acids
WBC	White Blood Cells
WHO	World Health Organisation

# KNUST



## DEDICATION

This thesis is dedicated to my parents, Engineer and Mrs. Mayowa Osunde for their financial and moral support.

# KNUST



## ACKNOWLEDGEMENT

My joy knows no bounds as I express my heartfelt appreciation to the magnanimous benevolent God for His unmerited grace and favour to his servant, and without Whom this research would have been incomplete.

Furthermore, I humbly express my heartfelt gratitude to my indefatigable supervisor, Prof. Daniel Boye Okai, for this great opportunity. Sir, God bless you for your counsel and efficacious impact in making me great. Thank you for your excellent instructions, encouragement, perseverance in morale upbringing and proof reading. My highest appreciation also goes to my co-supervisor, the amiable, articulate Dr. Michael Kwaku Boateng for his encouragement, stimulating suggestions, insightful prowess in directing me during my research, God bless you abundantly. My gratitude also goes to Prof. E.L.K. Osafo, who gave me the opportunity to be counted among all postgraduate students of the Department of Animal Science by assisting me through the rigorous process of admission. He was always there for me when I needed him. God bless you Sir, for all you have done for me.

I also owe a depth of thanks to all lecturers of the Department of Animal Science (KNUST), I say thank you for your wise counsels to students. My immense gratitude also goes to Mr. James G. Watson, the C.E.O of Basic Environmental Systems and Technology (BEST) Inc., Alberta, Canada for providing the financial and material support for the project. Dr. John Baah of Agriculture and Agri-Food Canada Research Centre in Lethbridge, Canada and Dr. Kwame Opong Anane (CEO of Oporhu Consultancy Ltd.) who is also the Country Director of Basic Environmental Systems and Technology (BEST) Inc., Ghana were very instrumental in the procurement of funds, and all the necessary logistics that ensured the completion of the project. I am greatly indebted to both of them and God bless them bountifully. I appreciate

my companion and research partner, Mr. Yaw Oppong Frimpong, for his encouragement, moral support and academic insight and without whom I would not have been able to complete my research. To Mr. L. Gariba, a staff of KNUST and Dr. D. Dei, the Chief Medical Director of Kumasi Veterinary Clinic, Amakom, Kumasi, I owe a debt of gratitude for their varied assistance. I also thank the vivacious Messrs. Joseph Larbi Agyapong and Bright Kwame Amponsah, both former national service personnel in the Department of Animal Science for their assistance during this research. I wish to thank the industrious, Mr. Faakye Nixon for his efficacious support. I can also not thank Mr. Prince Sasu, a student at the Department of Animal Science, KNUST enough for his help especially early in the morning. To all the workers of the Department of Animal Science (KNUST) who helped in various ways, I say a big Thank you.

I would also like to express my appreciation to Mr. and Mrs. S. Agyapong for their hospitality when I arrived in Ghana and my Pastors, David Adomako Danquah from C.A.C Zion Temple, Fumesua, and Nosakhare Okungbowa from C.A.C Soul Winning Centre, Nigeria. I also appreciate the support of Mr. and Mrs. K. Ibude and their family, my big brother Mr. Abel Okungbowa, my grandmother Madam Victoria Okungbowa and my uncles, Mr. Olu Okungbowa and Mr. Efosa Okungbowa. God richly bless you all, Amen.

# KNUST



## CHAPTER ONE

### 1.0. INTRODUCTION

The success of any animal production enterprise first and foremost depends on the feeding of well-balanced diets. Ghanaian pig farmers do not use well-formulated and compounded feeds and have concentrated on feeding pigs with whatever feedstuffs are at their disposal (Okai and Bonsi, 1989). This practice, which, to some extent, is due to the lack of knowledge of the nutritional needs of pigs during various phases of their life cycle has prevented most farmers from obtaining the maximum profit due to slow growth and poor rates of conversion of feed.

Also, the poor management practices in weaning, transportation and handling of livestock are stressful and can predispose these animals to several diseases and health related issues (Morrow, 2002). In some parts of the world, farmers resort to the addition of low doses of antibiotics to the feed of farm animals in an effort to promote growth so as to ensure that animals reach their required market weights in time and also to reduce the cost of production. Buchanan *et al.* (2008) also explained that sub-therapeutic doses of antibiotics are being used as a preventive measure against the outbreak of diseases. It has been stated that because of the intensive usage of antibiotics in animal production, some farmers are now of the notion that animal production will be impossible without the use of antibiotics (Witte, 1998).

The use and/or misuse of antibiotics in animal growth promotion has recently become unpopular due mainly to the fact that antibiotic residues may be present in animal products meant for human and animal consumption (Doyle, 2001; Gracey *et al.*, 1999). It has been speculated that some of these residues are partly responsible for the increases in the occurrence of antibiotic resistant strains of bacteria which are making years of research into drugs and monies spent go waste; and also increasing the incidence of bacteria related

epidemics and the number of deaths due to pathogens which are not easy to control. (Gracey *et al.* 1999).

Several countries including the United Kingdom (UK), Denmark and Sweden have therefore banned the use of antibiotic growth promoters (AGP) in animal production and have enacted strict legislations on the use of antibiotics in animal production. (Buchanan *et al.* 2008). In 2006, the European Union (EU) also banned in-feed, antibiotics used in animal production in all member countries.(Vondruskova *et al.* 2010). Furthermore, the United States of America (USA) has since December 2013 banned the use of all medically important antibiotics in animal production (FDA, 2013). Scientists have now intensified research into products that can effectively replace these in-feed antibiotics in growth and health promotion in animal production without being harmful to the health of humans and animals. Direct-fed microbial (DFM) or probiotics which, according to Fuller (1989), are viable microorganisms which improves the growth and health of farm animals have been tested by several researchers as a possible alternative to growth promoting antibiotics. Zani *et al.* (1998), for example, reported efficient utilization of feed and reduction in the incidence of diarrhoea in weanlings. De los Santos *et al.* (2005) also reported of a reduction in tumor growth in animal models when probiotics were administered. Again, the use of probiotics has been associated with an improvement in the microbial balance in the gut of farm animals (Gwendolyn, 2010) and production of short chain fatty acids (SCFA) which are responsible for inhibiting growth of pathogenic microflora (Roselli *et al.*, 2005). Matsuzaki and Chin (2000) have also reported that the use of probiotics improves the immune responses of farm animals and humans.

In Ghana, RE3<sup>TM</sup>, a DFM product produced and distributed by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada and its subsidiary in Ghana, has been observed to improve growth performance and efficiency in broiler and growing pigs (Bonsu *et al.*, 2012 ; Okai *et al.*, 2010); laying performance (Bonsu *et al.*, 2014) and reduce the cost

of production (Dei *et al.*, 2010). Basic Environmental Systems and Technology Inc. is on the verge of introducing 2 new products to the market but it is necessary to ascertain their effects on farm animals. Hence, this experiment was carried out to find out the effects of three DFM products produced by BEST Inc. (RE3<sup>TM</sup>, RE3 PLUS and a combination of RE3<sup>TM</sup> and P3) on the growth performance, gut microbial profile and carcass composition of growing pigs.

# KNUST



## CHAPTER TWO

### 2.0. LITERATURE REVIEW

#### 2.1. Feed Additives

There are several schools of thought on what feed additives are and depending on the context, the definition of feed additives may vary. Kamra and Pathak (1996) defined feed additives as substances of non-nutritive nature which are used in minute quantities for improving or stimulating growth and in some cases for preventing or controlling disease conditions and parasite infestation in farm animals. Hutjens (1991) and Lewis (2002) also defined feed additives as substances that are added to animal feed to improve its physical and chemical properties such as texture, flavour, pH, odour etc. so as to enhance the usefulness of the feed to the animal and at the same time reduce farmers' expenditure on feeding. It has however been emphasized that, some feed additives are actually added to animal feed to compensate or correct some nutritional deficiencies in animals or inadequacies that may be present in the feed being given to farm animals (Gillespie, 1998; Kellems and Church, 2002).

##### 2.1.1. Benefits of Feed Additives

Zimmerman (1986) explicated that, feed additives are more useful in growing animals and indicated through several literatures that, it has been found that feed additives can account for as much as 15% improvement in growth of starter pigs and an average of 6% improvement in feed efficiency. In older pigs however, an average of 4 and 2% improvement were reported in growth rate and feed conversion efficiency, respectively.

It has also been stated that feed additives, like enzymes, and organic acids, can be used to enhance the nutrient availability of feed (Wenk, 2000). Some feed additives such as organic

acids are also added to the diet of animals to modify its acidity so as to preserve and also enhance the utilization of the feed (Papatsiros *et al.*, 2012).

Other benefits of feed additives according to Pandey and Upadhyay (2012) include reduction in feed wastage through binding of powdered feed; improve acceptability of feed by enhancing texture, improving sweetness, improving odour, etc.; reducing toxicity by binding some of the toxins in feed and encouraging consumer acceptability of meat through colour modification.

### **2.1.2. Types of Feed Additives**

Though several systems of categorization of feed additives exist, the European Food Safety Authority (EFSA, 2003), classified feed additives used in animal production into 5 distinct groups. These groups are:

- i. Nutrient Additives- These are additives that are added to the diets of animals to supply some specific nutrients which may not be present or may not be in the required amounts. Nutrient additives may consist mainly of vitamin and trace mineral supplements which may be given to animals because they may not have access to their natural habitats where these nutrients may be in abundance. Furthermore, some essential amino acids may be supplied as additives in the diets of farm animals.
- ii. Sensory Additives- These are additives that stimulate the animals' appetite and therefore improve the voluntary feed intake of the farm animals. Most of these additives improve the flavour of the feed or may take away some odours that reduce feed acceptance. Examples of sensory additives include sweeteners, and colouring and flavouring agents.

- iii. Coccidiostats and Histomonostats- These are anti-protozoal agents that act on coccidia (parasites).
- iv. Zootechnical Additives- The function of zootechnical additives is not to provide the animal with nutrients but rather to enhance the efficient use of the nutrients supplied in the diet. Most zootechnical feed additives such as enzymes may improve efficiency by degrading complex feed nutrients into forms which are readily absorbable or by stimulating the immune system of animals e.g. phytobiotics/phytogenics or by a combination of both mechanisms (probiotics). Aside their effects on the animal, some additives in this group such as probiotics may also help reduce the harmful effects of environmental pollution that animal production may pose.
- v. Technological Additives- This group of feed additives helps in the handling of feed. Technological feed additives used in animal production include acidifiers, preservatives, binders, anti-caking agents, coagulants, anti-oxidants and acidity regulators.

Kamra and Pathak (1996) earlier classified feed additives into the following groups:

- i. Chemical compounds like arsenicals and copper sulphate
- ii. Tranquilizers
- iii. Surfactants
- iv. Antioxidants
- v. Antibiotics
- vi. Hormones (natural, synthetic and hormone-like substances)
- vii. Probiotics
- viii. Miscellaneous substances like colouring and flavouring agents, etc.

A simple system of classifying feed additives according to Banerjee (1988) is where feed additives are grouped based on whether they supply animals with nutrients or not. Thus, this system groups feed additives used in animal production into nutritive and non-nutritive feed additives. Nutritive feed additives as the name implies are additives that supplies the animal with nutrients whilst non-nutritive feed additives consist of all other additives that do not supply the animal with nutrients but are required for the smooth growth of the animal. Several non-nutritive feed additives have come under serious scrutiny and according to Stephany (2010) and Vondruskova *et al.* (2010), this has led to the ban on some of them, notably, antibiotics. Thus, the need arises to find suitable alternatives which are not harmful to the health of man and animals.

## **2.2. Antibiotics as Additives in Animal Feed**

The discovery of antibiotics was one of the world's major breakthroughs in science because it helped in the fight against infectious diseases caused by bacteria. Antibiotics literally meaning "against life" (Kellems and Church, 2002), and according to Gracey *et al.* (1999), are compounds produced wholly or partly by microorganisms such as molds and bacteria; possessing the ability to curb the growth of or to kill bacteria. The use of antibiotics in growth promotion in farm animals dates back to the late 1940's and early 1950's when Moore and his colleagues and Jukes and his colleagues observed growth stimulating responses upon the addition of streptomycin and aureomycin respectively to the diets of chicks (Buchanan *et al.*, 2008; Dibner and Richards, 2005). It has further been explained that because of the need to boost food production in the post-World War II era, several animal farmers started the extensive use of antibiotics in animal feeding which resulted in the occurrence of resistant strains of bacteria relating to animal production in early 1951 (Starr and Reynolds, 1951 as cited by Dibner and Richards, 2005). With time, antibiotic use in animal production became the second largest consumer of antibiotics in the world and some farmers even considered

animal production without the use of antibiotics as impossible (Witte, 1998). Thus, because of the high incidence of bacteria resistance and the ban on the use of antibiotics in all European Union (EU) countries and the ban on the growth promotional use of all medically important antibiotics in animal production in the United States, there will be the need to find suitable alternatives to these growth promoting antibiotics.

### **2.2.1. Mode of Action of Antibiotics**

It has been established that antibiotics exert their lethal effects on bacteria through 5 basic mechanisms which are achieved as a result of the presence and/or absence of certain structures or functions. Thus, Greenwood and Whitley (1997) stated that most of these antibiotics work on a concept of selective toxicity. The mechanisms by which these antibiotics work are the inhibition or interference with the synthesis of the cell wall, protein metabolic pathways and nucleic acid and the alteration of the cell membrane.

i. Alteration of the bacteria cell membrane permeability:

Antibiotics that are known to exhibit this mechanism are known to be bactericidal. They alter the cell membrane such that porins are too big to allow cell contents to leak out readily or are so small and do not allow the bacteria to obtain the necessary nutrition and nourishment (Bezoen *et al.*, 1999 ; Soares *et al.*, 2012). Greenwood and Whitley (1997) stated that the groups of antibiotics that exert this action probably do so by attacking the exposed phosphate groups of the cell membrane phospholipids. Antibiotics that are known to utilize this form of action are called ionophores.

ii. Interference with the metabolic pathway:

This group of antibiotics may stop the reproduction of bacteria without necessarily harming it. The main function of antibiotics that use this approach is to either tie up enzymes resulting in the blocking of important steps in metabolism or some of these drugs may resemble

substrates so much that the limited number of enzymes may not get the chance to work on all the substrates (Bezoen *et al.* 1999). Thus, instead of the reaction resulting in end products which may commence another process, the entire process comes to a halt. Sulfonamides and trimethoprim are noted to portray this action.

iii. Inhibition of nucleic acid synthesis

Antibiotics of the quinolones, novobiocins, rifampicins, diaminopyrimidines, sulphonamides and nitroimidazoles genera have been observed to kill bacteria cells by binding directly to the helix structure of the cell's deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) (Greenwood and Whitley, 1997; Soares *et al.* 2012) or by interfering with DNA-associated enzymatic processes.

iv. Interference with cell wall synthesis

The peptidoglycan wall around the bacteria is responsible for maintaining the structural strength of the bacteria (Greenwood and Whitley, 1997). Antibiotics of the  $\beta$ -lactam group kill gram positive and negative bacteria by inhibiting several processes in the formation of this wall. The peptidoglycan wall is therefore unable to support the osmotic pressure from both inside and outside of the bacteria cell (Soares *et al.* 2012).

v. Inhibition of protein synthesis

This mechanism adopted by some antibiotics is bacteriostatic in nature. Antibiotics, like the aminoglycosides and macrolides, stagnate the growth of bacteria by interrupting with the formation of the initiation complex required for the protein synthesis by binding to ribosomes within the bacteria.

Table 1 is a summary of some groups of antimicrobial agents, their effects on the bacteria and the mechanism of action which they employ.

Table 1: Some antibiotics, their effects and their mechanism of action.

Group of antimicrobial agent	Effect on bacteria	Mode of action
Penicillin	Bactericidal	Inhibition of cell wall synthesis.
Cephalosporin	Bactericidal	Inhibition of cell wall synthesis.
Carbanepems	Bactericidal	Inhibition of cell wall synthesis.
Polypeptide antibiotics	Bactericidal	Inhibition of cell wall synthesis.
Quinolones	Bactericidal	Inhibition of DNA synthesis.
Metronidazole	Bactericidal	Inhibition of DNA synthesis.
Rifamycins	Bactericidal	Inhibition of RNA transcription.
Lincosamides	Bactericidal	Inhibition of protein synthesis
Aminoglycosides	Bactericidal	Inhibition of protein synthesis
Macrolides	Bacteriostatic	Inhibition of protein synthesis
Tetracyclines	Bacteriostatic	Inhibition of protein synthesis
Chloramphenicol	Bacteriostatic	Inhibition of protein synthesis
Sulfonamides	Bacteriostatic	Competitive inhibition/ interference with metabolism.

Source: Byarugaba (2010)

### 2.2.2. Growth Promotion Effects of Antibiotics

Doyle (2001) has intimated that antibiotic growth promoters (AGP) which are low doses of antibiotics added to the feed of farm animals on daily basis improve the rate of growth in farm animals by ensuring that animals do not compete with any bacteria for nourishment. The author (Doyle, 2001) further stated that these antibiotics improve growth in farm animals by three (3) primary effects which are the improvement in the feed efficiency, increase in growth and lowering the incidence of diseases. According to Dibner and Richards (2005), the growth promoting effects of antibiotics were discovered when Moore and his colleagues were feeding by-products from the production of streptomycin to chicks as a source of vitamin B<sub>12</sub> but observed increases in growth which they could not explain. Later, Stokstad and Jukes, and Cunha and his colleagues in 1950 respectively observed significant improvement in the rate of gain in birds and pigs respectively fed diets containing aureomycin. Several antibiotics

have been used in growth promotion in farm animals and according to Witte (1998) this has resulted in the increase in resistant strains of bacteria which has been attributed partly to antibiotic residues found in meat and other animal products.

### **2.2.3. Antibiotic Resistance**

Dzidic *et al.* (2008) and Hooper *et al.* (2001) defined microbial resistance as an adaptation mechanism exhibited by bacteria and other microbes to all forms of biochemical stress. Thus, microbes in general have a way of defending themselves from the hazardous environment in which they find themselves. It has further been explained by Byarugaba (2010) that resistance to toxins and other antimicrobial substances by bacteria existed long before humans discovered antibiotics but antimicrobial resistance has become important recently due mainly to the fast rate at which bacteria and other microbes resist these antimicrobials, thereby rendering monies and time spent on research and drugs waste. The mechanisms by which bacteria resist antimicrobials according to Dzidic *et al.* (2008) and Hooper *et al.* (2001) include the following:

- i. Pumping out the antimicrobial substances from the bacteria.
- ii. Modification of cell wall permeability such that antimicrobials cannot enter to cause harm.
- iii. Modification of targets such that they are no longer bound by the antimicrobial substances.
- iv. Some bacteria also absorb insignificant quantities of these antimicrobial substances and are therefore not harmed by them.
- v. Some bacteria also produce enzymes which hydrolyse antibiotics before they reach the target sites.

- vi. It has also been stated that some bacteria may produce relatively more targets than what the dosage of antibiotics can bind and by so doing render the antibiotic ineffective.

#### **2.2.4. Forms of Resistance**

There are two (2) major ways by which bacteria acquire resistance to antimicrobials. These forms are the intrinsic resistance and the acquired resistance.

- a. Intrinsic resistance

As the name implies, this form of resistance exists as a result of the nature of the bacteria. Tenover (2006) emphasized that this is the form of resistance that informs scientists on the way in which the bacteria or microbe should be controlled. An example of intrinsic resistance is the ability of gram-negative bacteria to resist glycopeptides because their outer membranes are impermeable to the large molecule (Matthew *et al.* 2003). This form of resistance is what the bacterium uses to protect itself in its natural environment.

- b. Acquired resistance

Acquired resistance according to Bezoen *et al.* (1999), is the ability of a microorganism to resist the activity of an antimicrobial agent to which it was previously susceptible to. Acquired resistance can be grouped into two basic forms (Catry *et al.*, 2003). These forms are the mutation of chromosomes and the horizontal gene transfer.

Mutation of chromosomes is a form of alteration or change in the sequence of nucleotides in the DNA of a bacterium (Schleif, 1993). It has been stated that mutation is inheritable and it is essential in the survival of the bacteria (Hooper *et al.*, 2001). Bacteria cells mutate so as to attain a perfect state and also to reduce their susceptibility to harsh environmental conditions. Birošová and Mikulašová (2005) explained that this form of resistance can occur with or without the presence of an antimicrobial agent and therefore is not of major concern to

humans. Schleif (1993) explained that chromosomal mutation involves the modification of the amino acids of proteins which results in the changes in the expressions of genes. Mutation normally result in changes which will mean that previously bactericidal or bacteriostatic agents will no longer be effective since their targets may no longer be in existence or may have changed. Schleif (1993) further stated that mutation may affect the bacteria positively or negatively since it may improve or deteriorate its ability to resist antibiotics.

Unlike chromosomal mutation, horizontal gene transfer involves the picking up of functional DNA from other bacteria or from the environment (Bezoen *et al.*, 1999). Džidic *et al.* (2008) explicated that horizontal gene transfer is the form of resistance acquisition which is medically important because it has resulted from the use and/or misuse of antibiotics or antimicrobial substances. There are 3 forms of horizontal gene transfer. These are transformation, transduction and conjugation.

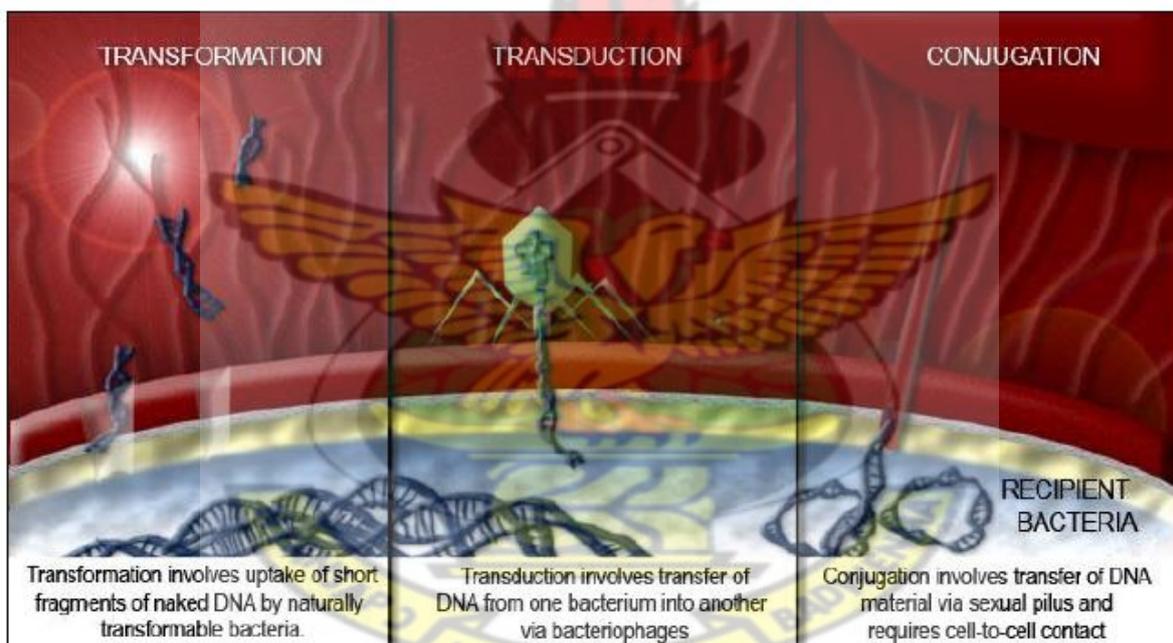
When bacteria die, they leave their DNA behind and other bacteria that are compatible with this DNA may pick them up and integrate them into their own DNA. This process, according to Bezoen *et al.* (1999), is called transformation. In situations where the DNA contains resistant chromosomes, then the bacteria may become resistant.

Transduction, on the other hand, occurs when a bacteriophage transfers chromosomes from one bacterium into another (Džidic *et al.*, 2008). Bacteriophages are viruses that attack bacteria by first introducing its DNA into the bacteria's cytoplasm. The DNA then disintegrates the bacteria's DNA and also multiply after which repackaging happens. In some situations, the bacteriophages may package only bacteria DNA and subsequently may transfer only bacteria DNA to their next host (Bezoen *et al.*, 1999). Resistance may then be acquired when the introduced DNA contains resistant genes which may be incorporated into the

bacteria's DNA. Džidic *et al.* (2008) further indicated that this form of horizontal gene transfer is not very important since bacteriophages have a narrow spectrum of hosts.

Conjugation, on the other hand, involves the transfer of DNA through direct cell to cell contact (Serna *et al.* 2010). Llosa *et al.* (2002) rather referred to conjugation as the promiscuous way by which DNA is transferred from one bacterium into another. This form of horizontal gene transfer is the most important since it can occur between closely related bacteria and unrelated bacteria.

Plate 1 gives a diagrammatic representation of bacteria transformation, transduction and conjugation.



Source: Msu (2011).

Plate 1: Diagrammatic representation of the 3 forms of horizontal gene transfer

### 2.3. Alternatives to Antibiotics in Animal Growth Promotion

Because of the increase in resistance and the outbreak of diseases caused by these resistant bacteria strains, it has become important to stop the use of medically important antibiotics in animal production and also to ban the use of AGP (Buchanan *et al.*, 2008). Thus, there has

been the need to find alternatives to AGP and antibiotics used in animal production and feeding.

Several strategies according to Doyle (2001) have been proposed among which the use of other feed additives is prominent. Doyle (2001) further indicated that, for any additive to effectively replace antibiotics in growth promotion, that additive should be able to improve feed efficiency, increase growth rate and also lower the occurrence of certain diseases. Adjiri-Awere and van Lunen (2005), Cromwell (2001), Hardy (2002), and Verstegen and William (2002) indicated that some feed additives that are being tested as possible alternatives to antibiotic growth promoters include:

- Acidifier or Organic Acids
- Dietary or Exogenous Enzymes
- Clay Adsorbents
- Phytobiotics or Botanicals
- Prebiotics and
- Direct-Fed Microbial (DFM) or Probiotics

### **2.3.1. Acidifiers or Organic Acids**

Mroz (2005) defined organic acids as carboxylic acids including amino and fatty acids of the general structure  $R-COOH$  which contain 1 to 7 carbon atoms. Organic acids or acidifiers which are employed in animal feeding include benzoic acid, citric acid, tartaric acid, fumaric acid and malic acid (Dibner and Buttin, 2002). Acidifiers have been observed to improve the health of farm animals, increase their ability to resist diseases and improve utilization of feed. Various modes by which acidifiers can exert these beneficial effects on farm animals have been proposed by Diebold and Eidelburger (2006), Knarreborg *et al.* (2002), Partanen and Mroz (1999), Partanen (2001), and Tung and Pettigrew (2006). These strategies include:

- i. Reduction of gastric pH
- ii. Reduction of buffering capacity of diets
- iii. Increase of proteolytic enzyme activity
- iv. Stimulation of digestive enzymes
- v. Improvement in nutrient digestibility
- vi. Promotion of beneficial bacteria growth
- vii. Direct killing of pathogenic bacteria
- viii. Alteration in the nutrient transport and synthesis within the bacterium
- ix. Depolarization of bacterial membrane.

The addition of acidifiers to the diet of weanlings has been discovered to reduce the incidence of scouring and post-weaning mortality (Tung and Pettigrew, 2006). Also, Tung and Pettigrew (2006) further reported of a 2.7% increase in the rate of growth in finishing pigs and 3-5% in growing pigs fed diets containing organic acids. Again, Boiling *et al.* (2000), Burnell *et al.* (1988), Eckel *et al.* (1992), Giesting *et al.* (1991) and Tsioloyiannis *et al.* (2001a and b) have reported improved performances in pigs fed diets supplemented with organic acids. Mroz (2005), however, indicated that the use of organic acids has been a deterrent to most farmers since its pungent smell does not encourage intake and also its corrosive nature damages feeding equipment.

### **2.3.2. Dietary or Exogenous Enzymes**

Robert *et al.* (2003) defined enzymes as organic catalyst that speed up the rate of biochemical reactions in living systems thereby making life possible. Enzymes are highly specific and according to Garrett *et al.* (1999), the relationship between enzymes and their host can be likened to a “lock and key mechanism” where specific enzymes work on specific substrates. Unlike other alternatives to AGP, exogenous enzymes work by breaking down complex nutrients which cannot be broken down by the animals’ own enzymes. Thus, Okai *et al.*

(2000) emphasized that enzymes improve the efficiency of conversion of feed by making the nutrients in the feed available to the animal. Unlike ruminants, monogastric farm animals are poor users of high fibrous diets and thus exogenous enzymes may be added to their feed to breakdown some of the complex polysaccharides in them so that they are readily absorbable or they are in forms which can be broken down further by the enzymes within the animal's gastrointestinal tract (GIT). Boateng *et al.* (2010) observed better feed to gain ratios and lower feed costs in pigs fed palm kernel cake-based diets and supplemented with an exogenous enzyme product known as; Mannanase PLT™.

### 2.3.3. Clay Adsorbents

The consumption of feed devoid of toxic substances improves performance and increases efficiency. This is the rationale upon which clay adsorbents work (Jacela *et al.*, 2010). Clay adsorbents mainly adsorb toxins which are produced by microorganisms. Unlike antimicrobials, clay adsorbents do not kill or retard the growth of bacteria, fungi and other pathogenic microbes but rather adsorb the toxins that these micro-organisms produce. It has been stated (Wan *et al.*, 2013; Wicklein *et al.*, 2008) that mycotoxins are harmful to the growth of farm animals, in that, they are mutagenic, immunosuppressive and carcinogenic. Galan (1996) indicated that the functions of clay adsorbents when added to the diets of farm animals include:

- i. Reducing excess acidity
- ii. Detoxification of diets by adsorbing toxins
- iii. Improving the palatability of the diet by adsorbing compounds responsible for unpalatability as well as antinutrients.
- iv. Reduction of bloat and diarrhoea in farm animals and

- v. Some clay adsorbents can also adsorb some microbes responsible for the production of toxins.

Several studies have reported of the importance of clay adsorbents in the growth and well-being of farm animals. In swine, Bartko *et al.* (1983), Brouillard and Rateau (1989), Castro and Elias (1978), Dominy *et al.* (2004), Martinez *et al.* (2004), Narkeviciute *et al.* (2002), Papaioannou *et al.* (2004) and Ramu *et al.* (1997) reported of reduced incidence of scouring upon the addition of clay adsorbent to diets.

#### **2.3.4. Phytobiotics**

Phytobiotics, also known as “plant extracts” or “botanicals” are chemical substances, obtained from plants, which are biologically active but not nutritive (Wenk, 2003). Wenk (2003) further indicated that some of these extracts from herbs and spices can have antimicrobial and even anti-helminthic activities. Also, reports indicate that phytobiotics may stimulate appetite in farm animals by directly improving feed palatability or by enzymic activity (Jones, 2002). Other functions of plant extracts include: improvement in immune response (Ilsley and Miller, 2005) and improving the composition of beneficial microbes (Lan *et al.*, 2004). It has been reported in some cases that phytobiotics may directly supply nutrients to animals (Zanu *et al.*, 2011).

In pigs, Ilsley *et al.* (2003) observed that the addition of a collection of plant extracts to the diets of lactating sows improved piglet performance prior to weaning. In a more recent study, Ilsley and Miller (2005) observed enhanced immune function in weanling pigs upon the supplementation of their diets with phytobiotics.

### 2.3.5. Prebiotics

The term prebiotics was first coined by Gibson and Roberfroid (1995) and they simply are the non-digestible food constituents which are potentially beneficial to the health of the host due to their fermentable properties which may stimulate the growth and activity of one or a limited number of salutary bacteria in the caecum. For an additive to be described as a prebiotic, it should possess three properties. These properties, according to Scantlebury-Manning and Gibson (2004) are:

- i. The ability to resist digestive enzymes within the stomach,
- ii. The ability to support the growth of salutary bacteria such as *Bifidobacteria* and
- iii. Products of their fermentation should not be injurious to the animal.

Gibson and Roberfroid (1995), Gibson (2004), Marinho *et al.* (2007), Rayes *et al.* (2009) and Verstegen and Williams (2002) indicated that some common prebiotics used in human foods and animal products include inulin, lactulose, oligofructose and galacto-oligosaccharides. Gibson and Roberfroid (1995) further indicated that the mechanism by which these prebiotics work include:

- i. The direct killing of pathogenic microbes through the production of bactericidal and bacteriostatic compounds.
- ii. The production of short chain fatty acids (SCFA) which are useful to some salutary microbes but toxic to some pathogenic microbes.
- iii. Some prebiotic substances modify the gut environment by increasing the acidity which may retard the growth of some pathogenic microbes.

Several researchers have reported beneficial effects that prebiotics may confer on farm animals. Houdijk (1998) and Smits (1996), for example, indicated improved growth

performance in weanlings fed diets supplemented with inulin and fructooligosaccharides (5-15g/day versus control 15-20g/day and 10-15g/day versus control 20-25g/day) respectively. (Gibson, 2004) reported that two varying levels of prebiotics, (4% containing fructooligosaccharides versus 1-4% control) were added to the diets of weanlings, where an improved average daily weight gain as compared to the control was observed.

### **2.3.6. Direct-Fed Microbials (DFM) or Probiotics**

Direct-Fed Microbials (DFM) or probiotics are simply viable microbes which affect the host in a beneficial manner by improving its intestinal microbial balance (Fuller, 1989). Guarner and Schaafsma (1998) however defined probiotics as “live microorganisms which upon consumption in certain quantities confer health benefits aside basic nutrition.” The FAO/WHO (2002) simply defined probiotics as viable cultures of microorganisms which when consumed in right proportions bestow health benefits on the host.

Probiotic products are available in diverse forms in the market. Some of these forms are capsules, liquid solutions, gels, boluses, powders, pastes, etc. The FDA (1998) indicated that unlike antibiotics, DFM is very effective during the weanling stages of pigs when the gut flora is changing due mainly to the exposure of pigs to solid feed.

#### **2.3.6.1. Microorganisms Used in DFM**

Probiotic microorganisms used mainly in human and animal nutrition are strains of bacteria and fungi. Fuller (1989) emphasized that lactic acid bacteria (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*, etc.), some bacteria from the *Bacillus* genus and some fungi of the *Saccharomyces* genus are the common ones used in the manufacture of probiotics but this notwithstanding, strains of *Escherichia coli*, and *Aspergillus* are also being used in the manufacture of probiotic products. The Association of American Feed Control Officials

(AAFCO, 1998) and the Food and Drug Administration (FDA, 1998) approved the species of microbes which can be used in the manufacture of DFM products. These species include:

- a. *Bacillus*: They are spore forming, gram positive bacteria that are static and have the ability to withstand a wide range of temperatures but grow well in acidic conditions.
- b. Lactic acid bacteria: Lactic acid bacteria are gram positive spore forming bacteria. They produce substances which include bacteriocins, antibiotics, lactic acid and peroxides (Lee *et al.*, 2009) which are harmful to pathogenic bacteria. Examples of lactic acid bacteria include species of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*.
- c. *Saccharomyces cerevisiae*: They are unicellular non-pathogenic fungi which are used mainly in the brewery and bakery industries.

The species of microorganisms approved by AAFCO (1998) and FDA (1998) for use in DFM products are as summarized in Table 2.

Table 2: FDA and AAFCO Approved Microorganisms for use in DFM products

<i>Aspergillus niger</i>	<i>Bifidobacterium infantis</i>	<i>Lactobacillus reuteri</i>
<i>Aspergillus oryzae</i>	<i>Bifidobacterium longum</i>	<i>Leuconostoc mesenteroides</i>
<i>Bacillus coagulans</i>	<i>Bifidobacterium thermophilum</i>	<i>Pediococcus acidilactici</i>
<i>Bacillus lentus</i>	<i>Lactobacillus acidophilus</i>	<i>Pediococcus cerevisiae</i> ( <i>damnosus</i> )
<i>Bacillus licheniformis</i>	<i>Lactobacillus brevis</i>	<i>Pediococcus pentosaceus</i>
<i>Bacillus pumilus</i>	<i>Lactobacillus bulgaricus</i>	<i>Propionibacterium</i> <i>freudenreichii</i>
<i>Bacillus subtilis</i>	<i>Lactobacillus casei</i>	<i>Propionibacterium shermanii</i>
<i>Bacteriodes amylophilus</i>	<i>Lactobacillus cellobiosus</i>	<i>Saccharomyces cerevisiae</i>
<i>Bacteriodes capillosus</i>	<i>Lactobacillus curvatus</i>	<i>Streptococcus cremoris</i>
<i>Bacteriodes ruminicola</i>	<i>Lactobacillus delbrueckii</i>	<i>Streptococcus diacetylactis</i>
<i>BactSeriodes suis</i>	<i>Lactobacillus fermentum</i>	<i>Streptococcus faecium</i>
<i>Bifidobacterium adolescentis</i>	<i>Lactobacillus helveticus</i>	<i>Streptococcus intermedius</i>
<i>Bifidobacterium animalis</i>	<i>Lactobacillus lactis</i>	<i>Streptococcus lactis</i>
<i>Bifidobacterium bifidum</i>	<i>Lactobacillus plantarum</i>	<i>Streptococcus thermophiles</i>

Source: Alliance Animal Health (1998).

Before a microorganism is considered in the manufacture of probiotics, it must possess several attributes. These attributes according to AAFCO (1998), FDA (1998) and Fuller (1989) are:

- i. The microorganism must exert beneficial effects on the host.
- ii. The microorganism must not be pathogenic or harmful to the host.
- iii. The microbe to be used in the manufacture of probiotics should be tolerant to secretions of the GIT.
- iv. The microbes should be able to colonize the gut.
- v. Microbe used should be gram positive even though gram negative strains of bacteria are being used in the manufacture of some probiotic products.
- vi. Also, it is important for a probiotic microbe to have the ability to attach to the intestinal lining of their host.

#### **2.3.6.1.1. Bacterial DFM**

Several strains of bacteria have been used in the manufacture of DFM or probiotics. These bacteria include strains from the lactic acid bacteria (LAB) group which include *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Lactococcus* etc. Strains of *Bacillus*, *E. coli* and *Propionibacterium* have also been used in the manufacture of probiotics. Several researches have reported of the beneficial effects some of these bacteria used in making probiotics which confer an improved health performance on farm animals and humans. Anuradha *et al.* (2005), for example, indicated that bacteria such as those of the *Lactobacillus* genera have been very effective in shaping immune responses in the host animal. Anuradha *et al.* (2005) further indicated that DFM containing strains of *Lactobacillus* can help in combating several infectious diseases.

Also, Shin *et al.* (2008) indicated that lactic acid producing bacteria have been found to produce bacteriostatic and bactericidal compounds like bacteriocins, antibiotics, lactic acids and peroxides. These substances are noted to help in their colonization of the intestinal mucosa and also help in preventing pathogen attachment to the mucosa. In broilers, for example, strains of *Enterococcus* have been observed to produce substances which inhibit the growth of *Clostridium* and *Listeria* (Shin *et al.*, 2008).

The uses of some of these bacteria in DFM products are under scrutiny and several reports have indicated that some of these bacteria can be obligate parasites. Besselink *et al.* (2008) for example indicated that the use of probiotics in critically ill people may be harmful and observed increased death rates in patients with acute pancreatitis upon the administration of probiotics containing multiple bacteria strains. Wikipedia (2014) further indicated that children administered with probiotics are more likely to develop sensitivity to allergens. Again, the administration of *Lactobacillus* to critically ill people has been shown to cause *Lactobacillus* related septicaemia.

*a. Paenibacillus polymyxa*

*Paenibacillus polymyxa*, a gram positive, facultative anaerobic, endospore-forming bacteria will be discussed in this review because of its use in this study. *P. polymyxa* is not normally used in animal production and/ or feeding but mainly used in crops farming and other horticultural practices because of its ability to fix nitrogen (Anuraj *et al.*, 2012). It has further been established that (Anuraj *et al.*, 2012) *P. polymyxa* promotes plant growth by suppressing some plant diseases. In strawberries, *P. polymyxa* have been found to be effective in controlling the growth of *Fusarium oxysporum* which are responsible for causing seedling blight (Helbig, 2001). Timmusk and Wagner (1999) also indicated that *P. polymyxa* can effectively control *Pythium aphanidermatum* and *Phytophthora palmivora* in *Arabidopsis*.

Though not a normal flora in animals' gut, *P. polymyxa* has been found to produce bactericidal and bacteriostatic compounds which can regulate the population of gut pathogenic microflora. Some of the compounds they produce include: polymyxin, fusaricidin, paenibacillin and lantibiotics (Anuraj *et al.*, 2012). In animal production, Lal and Tabacchioni (2009) indicated that *P. polymyxa* produces metabolites which have been proved to decrease pathogen colonization in farm animals, notably poultry. Ravi *et al.* (2007) earlier suggested that *P. polymyxa* has inhibitory ability against harmful human and animal pathogens such as *Vibrio sp.* *P. polymyxa* also produces organic acids and hydrogen as by-products which are very useful in changing the pH of the gut thereby rendering it not suitable for pathogen growth.

#### **2.3.6.1.2. Fungal DFM**

Fungi are one of the earliest microorganisms used in the food of man and animals (Fuller, 1995). They have been used in food industries in the making of beer, wine and bread. According to Anuradha *et al.* (2005) and Nicola (2010), strains of *Saccharomyces cerevisiae*, *Aspergillus niger*, *Aspergillus oryzae* and *Saccharomyces boulardii* have been used in the manufacture of probiotics but the most commonly used ones are *S. cerevisiae* and *S. boulardii*. Nicola (2010) explained that fungi used in the manufacture of DFM improve growth in animals by helping in the synthesis of vitamins, amino acids and minerals. Doyle (2001) further indicated that the addition of cultures of *S. cerevisiae* stimulates feed intake. Huber (1990) also stated that *Aspergillus oryzae*, when added to the diets of sheep, improves growth.

### 2.3.6.2. Mechanism of Action of DFM

Probiotic microbes confer beneficial health effects on their hosts by several mechanisms. These mechanisms, according to Agata *et al.* (2013), Edens *et al.* (1997), Fuller (1989), Fuller (1995), Hutjens (2007) and Rolfe (2000) include:

- i. Competitive exclusion: This mechanism involves the use of nutrients and space by the probiotic microbes in such a way that pathogenic microbes do not have access to their basic nourishment. Thus, this mechanism ultimately kills or drastically reduces the number of pathogens within the gut. This is achieved by the production of substances which are harmful to the pathogens, the modification of the gut pH and the large number of probiotic microbes which reduces the amount of attaching sites of these pathogens (Agata *et al.*, 2013 ; Edens *et al.*, 1997)
- ii. Production of organic acids: Probiotic microbes have been found to produce organic acids through the feed/food they ferment. These organic acids according to Fuller (1989) and Rolfe (2000) serve as a source of energy used by the animal and also inhibit the growth of some pathogenic microbes by reducing the pH of the gut. Some of the organic acids produced by these microbes include acetic, formic and organic acids.
- iii. Production of antimicrobial substances: Some strains of probiotic microbes have been observed to directly kill or retard the growth of other microbes by producing bactericidal and/or bacteriostatic compounds such as antibiotics, bacteriocins and peroxides. For example, Ravi *et al.* (2007) explained that *P. polymyxa* produces antimicrobial compounds like polymyxin, fusaricidin and paenibacillin.
- iv. Enzymatic activity: Probiotic microbes have been noted to secrete enzymes which improve digestion by breaking down the complex carbohydrates which cannot be degraded by the endogenous enzymes produced by the host. The enzymes

produced by these salutary microbes, according to Rolfe (2000), are responsible for enhancing digestion in the host by interfering with metabolic activities of pathogens and also repairing some damaged cells within the GIT of the host.

- v. Stimulation of the immune system: The activity of the immune system is said to increase upon the addition of DFM to the diet of humans and farm animals.
- vi. Reduction in toxins: Pathogenic microbes produce toxins which can be harmful to animals and humans. These toxins may be responsible for the diarrhoeal diseases and even some carcinogenic conditions which occur in humans and animals. Some probiotic microbes especially the lactic acid producing bacteria are noted to reduce or detoxify these toxic substances.

#### **2.3.6.2. Effects of DFM**

Direct-fed microbials have been found to exert several beneficial effects on humans and animals when they are consumed. These effects according to Fuller (1989) and Rolfe (2000) include their effects on the gastrointestinal microflora, growth performance, immune system and nutrient synthesis and digestibility.

- i. Effects of DFM on the gastrointestinal microflora

The guts of newborn animals get colonized naturally by microorganisms from their dam and the environment (Hume *et al.*, 2003). Hume *et al.* (2003) further indicated that these organisms consist of beneficial as well as undesirable or pathogenic ones. Under suitable conditions, there is a balance between the beneficial bacteria and pathogenic microorganisms and the beneficial organisms in the gut establish a variety of symbiotic relationships with the host (Jin *et al.*, 1997). However, in situations of compromised hygienic conditions, drastic changes in diet, stressful conditions and after treatment with therapeutic antibiotics (Jin *et al.*, 1997), a scenario where pathogens outgrow and colonize the gut occurs.

Direct-fed microbial products which consist of large quantities of beneficial or commensal microbes are therefore added to the diets of these animals to enhance the composition of beneficial microorganisms in the gut of farm animals thereby restoring the balance. Macpherson and Harris (2004) explained that these commensal microorganisms that are added to the diets of farm animals are responsible for several activities including, the enzymatic breakdown of complex food substances such as cellulose and other non-starch polysaccharides (NSP) resulting in increases in the energy uptake of the animal. It has also been indicated that some of these commensal bacteria and fungi are responsible for the changes in the morphology and physiology of the gut which leads to increases in the surface area of the intestinal lining resulting in increase in absorption of nutrients (Shirkey *et al.*, 2006). The exposure of farm animals to these salutary microbes is also helpful in improving gut immunity since Macpherson and Harris (2004) observed a reduction in the number of IgA-producing plasma cells in the lamina propria of germ-free animals in comparison to those exposed to these commensal microbes.

ii. Effects of DFM on growth performance

The addition of DFM to the diets of animals ameliorates intestinal health which leads to better health and productivity. For example, Davis *et al.* (2008) indicated that the addition of (0.01% of  $2 \times 10^8$  control versus 0.05% of  $1.47 \times 10^8$  *Bacillus licheniformis* and *Bacillus subtilis* based-DFM resulted in improved average daily gain (ADG) of (0.1 versus control 0.062) in growing and finishing pigs. The addition of probiotics has also been indicated to be responsible for the increase in appetite and its resultant increase in feed intake. Anukam *et al.* (2005) explained that probiotics stimulate appetite in farm animals through the enzymes that they produce. Giang *et al.* (2010a and b) reported that the addition of lactic acid complexes comprising combinations of strains *Enterococcus faecium* 6H2 ( $3 \times 10^8$  CFU g<sup>-1</sup> versus control  $5 \times 10^8$  CFU g<sup>-1</sup>), *Lactobacillus acidophilus* C3 ( $4 \times 10^6$  CFU g<sup>-1</sup> versus control  $6 \times$

$10^6$  CFU g<sup>-1</sup>), *Pediococcus pentosaceus* D7 ( $3 \times 10^6$  CFU g<sup>-1</sup> versus control  $5 \times 10^6$  CFU g<sup>-1</sup>), *Lactobacillus plantarum* 1K8 ( $2 \times 10^6$  CFU g<sup>-1</sup> versus control  $4 \times 10^6$  CFU g<sup>-1</sup>) and *Lactobacillus plantarum* 3K2 ( $7 \times 10^6$  CFU g<sup>-1</sup> versus control  $9 \times 10^6$  CFU g<sup>-1</sup>) improved ( $P < 0.05$ ) daily feed intake and weight gain of pigs as compared to the control. Ross *et al.* (2010) also, reported better feed conversion efficiencies in pigs fed diets containing cultures of *E. faecium* ( $3 \times 10^8$  CFU g<sup>-1</sup> versus control  $5 \times 10^8$  CFU g<sup>-1</sup>). Again, Abe *et al.* (1995), Hong *et al.* (2002), Jasek *et al.* (1992) and Jonsson and Conway (1992) observed better weights gains in weanling, growing and finishing pigs fed *Lactobacilli*-supplemented diets. Matthew *et al.* (1998) reported improvement in weight gain when pigs were fed *Saccharomyces cerevisiae*-supplemented diets.

### iii. Effects of DFM on the immune system

Probiotics have been noted to stimulate the immune system by increasing cytokine expression, stimulating phagocytotic activity of white blood cells and increasing the production of secretory immunoglobulins through their enzymatic activity (Sanders, 2000). Others have indicated that probiotics arouse the activities of natural killer cells (Lessard and Brisson, 1987; Matsuzaki and Chin, 2000; Roselli *et al.*, 2005; Shu *et al.*, 2001). Wu (2006) however suggested that probiotics influence the immune responses by increasing the concentration of macrophage and phagocytic activity of peripheral blood monocytes and granulocytes and white blood cells.

Report by Takahashi *et al.* (1998) and Vitini *et al.* (2000) indicated that *Bifidobacterium longum* increase the activity of intestinal IgA. *L. plantarum* has also been found to enhance antibody activity against *E. coli* (Herias *et al.*, 1999). Other researchers have reported increases in the concentration of CD8+ T cells (Scharek *et al.*, 2007), lymphocytes (Szabo *et al.*, 2009) and IgA and IgM (Isolauri *et al.*, 2001).

#### iv. Effects of DFM on nutrient synthesis and digestibility

Probiotic addition to the diet of animals has been shown to be responsible for the improvement in the digestion and synthesis of nutrients. Fuller (1989) and Santos *et al.* (2005) explained that DFM enhances the utilization and synthesis of nutrients by animals by reducing the competition between the host (animals) and other moribific microflora within the gut of farm animals.

Also, the fermentative activity of some probiotic microbes have been observed to yield some volatile fatty acids (VFA) and vitamins which are also needed in the growth of farm animals (Playne, 2003). Playne (2003) further stated that probiotic microbes enhances the efficiency of utilization of feed by farm animals by producing enzymes which ensure the breakdown of feed substances like complex non-starch polysaccharides (NSP) and increasing the nutrient availability. Friend and Shahani (1984) had earlier indicated that probiotic supplementation improves the digestibility of dietary nutrients such as the carbohydrates, fats and proteins.

#### **2.3.7. RE3™ as a DFM Product**

RE3™ is a DFM product produced and distributed by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada. RE3™ is in the liquid form and is added to the diet, mixed thoroughly before being offered to the animals. The composition of RE3™ is as shown on Table 3.

Table 3: Composition of RE3™

<b>Constituents</b>	<b>Amount</b>
Water	99.9%
<b>Microorganisms</b>	
<i>Lactobacillus</i> sp.	1 x 10 <sup>8</sup> CFU/g
<i>Bacillus</i> sp.	4 x 10 <sup>12</sup> CFU/g
<i>Saccharomyces cerevisiae</i>	11 x 10 <sup>5</sup> CFU
<b>Minerals</b>	
Calcium	< 0.02 %
Sodium	< 0.02%
Potassium	< 0.005%
Magnesium	< 0.003%
Molybdenum	< 0.3ppm
Copper	< 0.3ppm
Iron	< 3ppm
Boron	< 3ppm
Zinc	< 2ppm

Source: Amoah (2010).

Several researches have been done in Ghana on the effects of RE3™ on different farm animals. Most of these researches have reported the beneficial effects that RE3™ can have on farm animals. Okai *et al.* (2010), for example, observed significant ( $P < 0.05$ ) improvements in average daily gain (ADG) ,feed conversion ratio (FCR) in weanling pigs and reported that pigs on 1ml/kg RE 3™ spent the shortest time, (113.8 days versus control 122.0days). In broilers, Bonsu *et al.* (2012) and Dei *et al.* (2010) observed better FCR upon RE3™ supplementation of (2.49 versus control 2.74). Bonsu *et al.* (2012) reported a reduction in serum cholesterol of RE3™ in broilers (91.25mg/dl versus control 110.25mg/dl). Furthermore, Dei *et al.* (2010) indicated that the addition of RE3™ to the diet of broilers reduced cost of production since there was no need for medicaments such as coccidiostats. Also, Osei *et al.* (2013) explained that RE3™ supplementation in gestating rabbits resulted in heavier bunnies. Wallace *et al.* (2012) also indicated that the addition of RE3™ to rabbit diets resulted in significantly ( $P < 0.05$ ) better FCR of (4.739 versus control 5.062), higher

white blood cell (WBC) and lymphocyte levels respectively ( $13.33 \times 10^9 \mu\text{L}$  and  $8.37 \times 10^3 \mu\text{L}$  versus control  $6.97 \times 10^9 \mu\text{L}$  and  $3.73 \times 10^3 \mu\text{L}$ ). The addition of RE3™, again, resulted in higher levels ( $P < 0.05$ ) of blood platelet in birds (Agyarko, 2013). Recently, Bonsu *et al.* (2014) reported that the addition of RE3™ to layer chicken diets resulted in the laying of heavier eggs with a reduction ( $P < 0.05$ ) in feed consumption as compared to the control.

#### **2.4. Summary of Literature Reviewed**

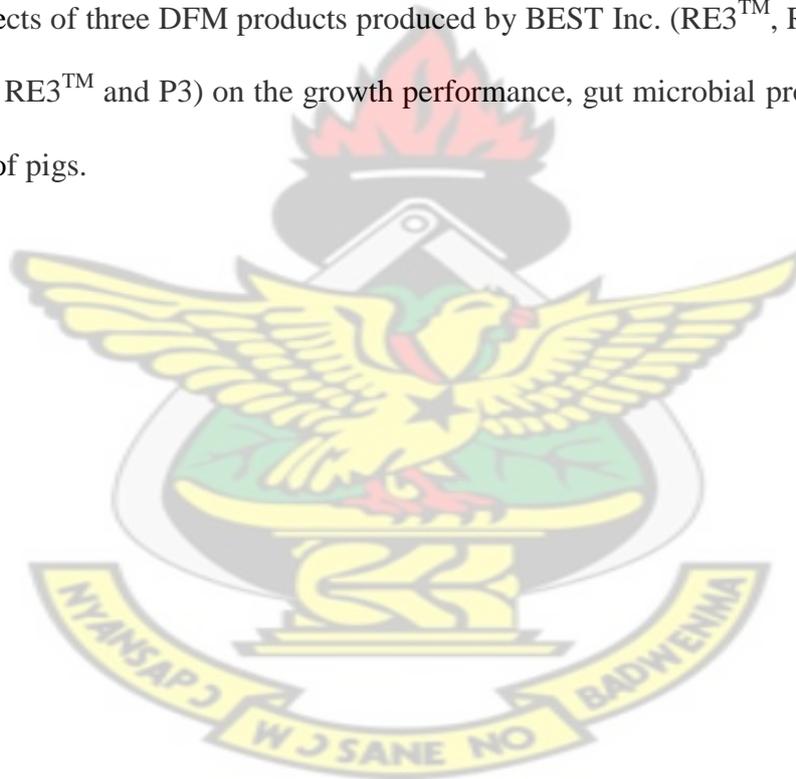
Several feed additives have been used to promote growth and well-being in farm animals but antibiotics were used extensively for this purpose. The rationale for adding antibiotics to the feed of farm animals was to provide a near germ-free state thereby resulting in a reduction in the competition between farm animals and pathogenic enteric microflora for their basic nourishment.

However, the use and/misuse of antibiotics in animal production, among other things, has led to the increase in resistant strains of bacteria which are responsible for several infections and epidemic with their resultant increases in mortalities in humans and animals due to the inability of drug to cure some of these diseases. Again, some residues of antibiotics used in animal production which may be potential allergens to some people have been found in the food of humans. Thus, these revelations led to a revolution against the use of antibiotics in growth promotion in farm animal. The EU, for example, has banned the use of all growth promotional antibiotics and requires several stringent procedures to be followed before even sick animal are administered with antibiotics. The United States, on the other hand, has banned the growth promotional use of medically important antibiotics in animal production.

However, because of the increase in demand for animal products, there has been the need to find suitable and sustainable alternatives which can successfully replace antibiotics in promoting growth without compromising the health of humans and animals. Probiotics or

DFM are one of such product that has been considered by scientists to replace antibiotic growth promoters. Direct-fed microbials have been observed to improve the growth, immune responses, gut microbial composition, digestibility, etc. in humans and animals.

RE3<sup>TM</sup>, a DFM product produced and distributed by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada and its subsidiaries in Ghana, have been observed to improve growth performance, efficiency, laying performance and reduces the cost of production. BEST Inc. is on the verge of introducing 2 new products to the market but it is necessary to ascertain their effects on farm animals. Hence, this experiment was carried out to find out the effects of three DFM products produced by BEST Inc. (RE3<sup>TM</sup>, RE3 PLUS and a combination of RE3<sup>TM</sup> and P3) on the growth performance, gut microbial profile and carcass characteristics of pigs.



## CHAPTER THREE

### 3.0. MATERIALS AND METHODS

#### 3.1. Site, Duration and Phases of the Experiment

The study was conducted at the Livestock Section of the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi and covered a period of twenty-two weeks (January to June 2013). The experimental area had mean monthly minimum and maximum temperature values of 22.25 and 33.33°C, respectively and a minimum and maximum relative humidity of 52.8% and 79% respectively. The average rainfall recorded during the experimental period was 1112.6mm. The study was divided into 2 phases: a starter phase where pigs were raised to a live weight of  $20 \pm 0.5\text{kg}$  and a grower-finisher phase where pigs were fed to a target body weight of  $70 \pm 0.5\text{kg}$ . The starter phase and grower-finisher phase lasted a period of 6 and 16 weeks respectively. Pigs were slaughtered for carcass analysis upon attaining the targeted body weight of  $70 \pm 0.5\text{kg}$  in the grower-finisher phase.

#### 3.2. Animals and Design of the Experiment

Twenty (20) Large White weanling pigs (12 females and 8 males) of an average initial weight of 9.4 kg were randomly allocated to four dietary treatments namely:

T1- Control (Basal diet with no added DFM).

T2- Basal diet + 1ml RE3<sup>TM</sup> per kg feed

T3- Basal diet + 1ml RE3 PLUS (a fermented product of RE3<sup>TM</sup>) per kg feed

T4- Basal diet + 1ml RE3<sup>TM</sup> and 0.5ml P3 (*P. polymyxa* based DFM) per kg feed.

The experiment was laid out in a Completely Randomised Design (CRD) and there were 5 replicates per treatment. Each replicate consisted of a single pig. There were 3 females and 2 males in each treatment.

### 3.3. Housing

The pigs were housed individually in concrete-floored wire mesh cages measuring 160 x 65 x 103cm which were located in roofed pens each measuring 365 x 315 x 100cm. Each pen had four of the individual cages. Wooden feed troughs measuring 23 x 12 x 8cm and 46 x 23 x 13 cm were used for the starter and grower-finisher phases respectively and each feed trough had wooden battens across the top to reduce feed wastage. Concrete water troughs measuring 23 x 12 x 10cm were provided in each cage.

### 3.4. Feeds and Feeding Regimes

Pigs were provided with *ad lib* access to a 23% CP diet (Table 4) for the starter phase. The feed was then changed into an 18% CP diet after the pigs had attained the targeted body weight of  $20 \pm 0.5$  kg for the grower-finisher phase. The DFM products were added to the feeds for pigs on treatments T2, T3 and T4 before they were offered.

Table 4: Percentage composition of the experimental diets.

INGREDIENTS	Creep-Starter	Grower-Finisher
Maize	54	58
Wheat bran	10	23.5
Soyabean meal	24	11.5
Fish meal	10	5
Vit/min premix <sup>o</sup>	0.25	0.25
Common salt	0.25	0.25
Oyster shell	1	1
Dicalcium phosphate	0.5	0.5
<b>Total</b>	<b>100</b>	<b>100</b>
<b>Chemical composition, calculated.</b>		
Crude protein (%)	23.02	18.02
Digestible energy (kcal/kg)	3305.60	3195.04
Calcium (%)	0.99	0.83
Phosphorus (%)	0.78	0.80

<sup>o</sup> Vitamin premix per kg diet: Vitamin A( $8 \times 10^5$  U.I); Vitamin D3 ( $1.5 \times 10^4$  U.I); Vitamin E (250mg); Vitamin K (100mg); Vitamin B2( $2 \times 10^2$ mg); Vitamin B12 (0.5mg); Folic acid (50mg); Nicotinic acid( $8 \times 10^2$ mg); Calcium panthotenate (200mg); Choline ( $5 \times 10^3$ mg).Trace elements:Mg( $5 \times 10^3$ mg); Zn( $4 \times 10^3$ mg); Cu( $4.5 \times 10^2$ mg); Co(10mg); I (100mg); Se(10mg). Antioxidants: Butylated hydroxytoluene( $1 \times 10^3$ mg).Carrier: CaCO<sub>3</sub> q.s.p (0.25kg).

### **3.5. Health**

Two incidences of mortality were recorded during the experiment and post-mortem examination was carried out at the Kumasi Veterinary Clinic, Amakom.

### **3.6. Management**

The pens and troughs were cleaned thoroughly and disinfected before the commencement of the experiment. Pigs to be used for the experiment were dewormed with multibendazole oral suspension<sup>1</sup> and identified with plastic ear tags. Water was provided *ad libitum*.

### **3.7. Parameters Measured**

#### **3.7.1. Feed Intake**

Feed offered weekly and weekly leftover feed was measured using a Camry Scale<sup>2</sup>. The difference between the feed offered and the leftover was considered to be the amount of feed consumed by the pigs. The average daily feed intake by pigs was also computed for as the ratio of the total feed consumed weekly to the number of days (7).

#### **3.7.2. Live Weight Changes and Weight Gains**

Pigs were weighed individually before the start of the experiment and subsequently on weekly basis using a Gascoigne Precision Scale<sup>3</sup>. Total weight gain was calculated by subtracting the final weight of a pig from its initial weight whilst the daily weight gain was calculated by dividing the weight gained by a pig by the number of days it spent on the experiment.

---

<sup>1</sup> Multibendazole oral suspension: Each ml contains Albendazole 25mg. Dosage: 2ml per 10kg bodyweight. Manufactured by Hebeiyuanzheng P harmaceutical Co. Ltd. Made in China.

<sup>2</sup> Camry scale (50g x25kg): Made in China.

<sup>3</sup> Gascoigne Precision Scale (200kgx500g): Manufactured by Precision Weighers, Reading, England.

### 3.7.3. Feed Conversion Ratio (FCR)

The efficiency of gain was calculated as the ratio of total feed consumed to the total weight gained by each pig.

### 3.7.4. Feed Cost and Economy of Gain

The cost of feed was calculated using the prevailing prices of the feed ingredients on the open market. The costs of the DFM products used were added to treatments T2, T3 and T4 and feed cost per kg weight gain was calculated by multiplying the cost of a kg of feed by the FCR.

### 3.7.5. Carcass Evaluation

The carcass parameters that were determined in the study were:

- i. **Dressed weight and dressing percentage:** The dressed weight of pigs was determined after slaughtering and evisceration. The dressed weight was determined as the weight of the eviscerated carcass with the head and trotters. This dressed weight served as basis for calculating the dressing percentage. Dressing percentage was inferred as the percentage of the ratio between the dressed weight and the final weight of the pig.

$$\text{Dressing percentage} = \frac{\text{Dressed weight of pig}}{\text{Live weight of pig}} \times 100$$

- ii. **Absolute and relative weights of viscera:** The absolute weight of the viscera was determined as the weight of the entire viscera while the relative weight of the viscera was calculated as the ratio of the weight of the viscera to the live weight of the pig x 100.

- iii. **Absolute and relative weights of respiratory tract:** The absolute weight of the respiratory tract was determined as the weight of the larynx, the trachea and lungs whilst the relative weight was determined as a percentage of the ratio of the weight of these organs to the live weight of the pig x 100.
- iv. **Absolute and relative weights of GIT (full and empty):** The weight of the GIT along with its contents was determined and weighing was done again after the contents had been emptied. Developments of the full and empty GIT were calculated subsequently.
- v. **Absolute and relative weights of empty stomach:** The weight of the stomach after its contents have been emptied was determined. The relative weight was also determined as the ratio of the weight of the empty stomach to that of the live weight of the pig.
- vi. **Absolute and relative weights of the liver, spleen, heart, kidney, trotters and head:** The weights of the liver, spleen, heart, kidney, trotters and head were all measured and these weights served as basis for calculating the development of the aforementioned components of each carcass.

Each carcass was then stored in a cold room at a temperature of 4°C for 24 hours after which the following parameters were taken:

- vii. **Chilled weight:** This was measured as the weight of the eviscerated carcass excluding the head and trotters.
- viii. **Carcass length:** The carcass length was determined as the distance between the forward edge of the first rib and the aitch bone.
- ix. **Mean backfat thickness:** The mean backfat thickness was calculated as the average of the backfat thickness measured at the first and last ribs and the last lumbar vertebra.

- x. **Loin eye area:** The Cross Section of the *Longissimus dorsi* muscle (sectioned between the 12<sup>th</sup> and 13<sup>th</sup> ribs) was traced out and scanned onto a computer. The area of the scanned images were then determined using AutoCAD Land Desktop 2009 version.
- xi. **P2:** The P2 was determined by measuring the backfat thickness, 6.5cm from the dorsal midline to the anterior portion of the last rib.
- xii. **Absolute and relative weights of the shoulder, belly, thigh and loin:** The weights of the shoulder, belly, thigh and loin were measured and their relative weights were determined as a ratio of these components to the final live weight of the pig x 100.

### 3.8. Faecal Microbial Analysis

Faecal samples were collected directly from the rectum of each pig during the grower-finisher phase into 10 ml plastic containers. These samples were then serially diluted before they were inoculated into a plate count agar (PCA). Samples were then incubated at a temperature of 35°C for 24 hours before a total viable count was done with the aid of a colony counter.

### 3.9. Statistical Analysis

All data collected were subjected to the analysis of variance (ANOVA) technique described in the Genstat 12<sup>th</sup> Edition (2009) and the differences between treatment means were separated using the least significant differences (LSD). However, missing data was analyzed using unbalanced analysis of variance (ANOVA) described in the Genstat 12<sup>th</sup> Edition (2009).

## CHAPTER FOUR

### 4.0.RESULTS AND DISCUSSION

# KNUST



# KNUST



---

---

---

---

---

# KNUST



---

---

# KNUST



# KNUST



---

---

---

---

# KNUST

---



# KNUST



---

---

---

---

# KNUST



---

---

---

---

---

# KNUST



---

---

---

---

---

---

---

# KNUST



# KNUST



# KNUST



## CHAPTER FIVE

### 5.0. CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that, generally the addition of the different DFM products to the diets of pigs did not seem to influence the growth performance, gut microbial composition and carcass characteristics of pigs. It is worth stating that the addition of RE3™ + P 3 resulted in larger liver sizes. It is recommended that further studies be undertaken to confirm the results obtained here and also to specifically delve more into the histological and pathological effects of the use of these new DFM products. In this respect, the need for on-farm studies cannot be over emphasized in view of the limitations of on- station research vis-à-vis, real production and productivity.



## 6.0. REFERENCES

- Abe, F., Ishibashi, N. and Shimamura, S. (1995).** Effect of administration of *Bifidobacteria* and lactic acid bacteria to newborn calves and piglets. *Journal of Dairy Science*. 78(12):2838– 2846.
- Adjiri-Awere, A. and Van Lunen, T.A. (2005).** Subtherapeutic use of antibiotics in pork production: Risks and alternatives. *Canadian Journal of Animal Science*. 85: 117-130.
- ADM Alliance Nutrition, Inc.** Available at: <http://www.admani.com/Animal%20Health/Tech%20Bulletins/Animal%20Direct%20fed%20microbial.htm> (Accessed 22/10/13).
- Adusah, A. (2009).** The effects of DFM on growth performance and carcass characteristics of starter pigs. B.Sc. Thesis, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, 80pp
- Agata., D, Izabella, K. and Marek, B. (2013).** Probiotics, prebiotics and synbiotics in poultry. Mode of action, limitation and achievements. *Journal of Central European*
- Agyarko, C. (2013).** Effects of DFM as administered in water on the health and performance of poultry in hot humid environment MSc. Thesis, KNUST, Kumasi. 102 pp.
- Amoah, K. O. (2010).** Effects of RE-3, a direct-fed microbial (DFM) product on the growth performance, blood profile and carcass characteristics of pigs. MSc. Thesis, KNUST, Kumasi. 104pp.
- Anukam, K. C., Osazuma, E.O. and Reid, G. (2005).** Improved appetite of pregnant rats and increased birth weight of newborns following feeding with probiotics *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14. *Journal of Applied Research*, 5(1): 46-52.
- Anuradha, S. and Rajeshwari, K. (2005).** Probiotics in health and disease. *Journal, Indian Academy of Clinical Medicine*, 6(1):67-72.
- Anuraj, N. S., Sheaza, A., Mukesh, Y., Sneha, S., Meer, A., Mayura, M. and Urvashi, P. (2012).** Identification and Characterization of neutral protease producing *Paenibacillus polymyxa* species EMB5024 by 16S r RNA Gene sequencing. *International Journal of Microbiology Research*, 4: 236-239.
- Apgar, G.A., Kornegay, E.T., Lindermann, M.D. and Wood, C.M. (1993).** The Effect of feeding various levels of *Bifidobacterium globusurn* A on the performance, gastrointestinal measurements and immunity of weanling pigs on the performance and carcass measurements of growing finishing pigs. *Journal of Animal Science* 71: 2173-2179.
- Association of American Feed Control Officials, (AAFCO) (1998).** AAFCO Official Publication. Atlanta: Georgia Dept. of Agric., USA. pp. 307-308.
- Banerjee, C. C. (1988).** Feed and Principles of Animal Nutrition. Oxford and I. B. H. Publishing Co. PVT. Ltd, New Delhi, Bombay, Calcutta, pp. 630-640.

**Bartko, P., Chabada, J., Vrzgula, L., Solar, I. and Blazovsky, J. (1983).** The fortification of pig ration with Zeolite in the cage rearing system ( in Czech). *Verterinarni Medicina* 28: 429-435.

**Besselink, G.H.M., Hjalmar, C.V.S., Erik, B., Marja, A.B., Harry, V.G., Harro, M.T., Vincent, B.N., Thomas, I.B., Bert, V.R., Ben, J.M.W., Camiel, R., Rutger, J.P., Menno, A.B., Alexander, F.M.S., Cornelis, H.C.D., Peter, J.W., Cees, J.H.M.V.L., Erwin, V.D.H., Casper, H.J.V.E., Miguel, A.C., Louis, M.A.A. and Hein, G.G. (2008).** Probiotic prophylaxis in predicted severe acute pancreatitis: a randomized, double-blind, placebo-controlled trial. Vol 371 pp651-659.

**Bezoen, A., van Haren, W. and Hanekamp, J.C. (1999).** Emergence of a debate: AGPs and public health. Human health and antibiotic growth promoters (AGPs): Reassessing the risk. HAN, Heidelberg Appeal Nederland Foundation. pp 7-89.

**Birošová, L. and Mikulašová, M. (2005).** Development of ciprofloxacin resistance due to mutations induced by 2-nitrofluorene. *Biomedical papers of the Medical Faculty of the University Palacký, Olomouc, Czechoslovakia*, 149 (2) 401-403.

**Boateng, M., Okai, D.B., Baah, J., Donkoh, A. and Mangalindan, J.F. (2010).** The growth performance and carcass characteristics of growing-finishing pigs fed palm kernel cake-based diets supplemented with Mannanase PLT™, an exogenous enzyme. Proceedings of the 1<sup>st</sup> Nigeria International Pig. Summit, 2010, Ibadan, Nigeria. P.93-98.

**Boiling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M. and Baker, D.H. (2000).** The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. *Journal of Animal Science* 78:682-689.

**Bonsu, F. R. K., Donkoh, A., Osei, S. A., Okai, D. B. and Baah, J. (2012).** Effects of direct-fed microbial and antibiotics supplementation on health status and growth performance of broiler chickens under hot humid environmental conditions. *International Journal of Livestock Production*, 3(6): 66-71.

**Bonsu, F. R. K., Donkoh, A., Osei, S. A., Okai, D.B. and Baah, J. (2014).** Direct-fed microbial on the health status, productive performance and internal egg characteristics of layer chickens under hot humid environmental conditions. *African Journal of Agricultural* 9(1):14-20.

**Brouillard, M.Y. and Rateau, J.G. (1989).** Adsorption of bacterial enterotoxins by smectite and Kaolin clays In Vitro study in cell- culture and in the new born mouse (in French). *Gastroenterologie Clinique et Biologique* ,13: 18-24.

**Brown, M. M. (2009).** The effects of DFM on growth performance and carcass characteristics of starter-grower pigs. B.Sc. Thesis, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, 103 pp.

**Buchanan, N. P., Hott, J. M., Cutlip, S. E., Rack, A. L., Asamer, A. and Moritz, J. S. (2008).** The effects of a natural antibiotic alternative and a natural growth promoter feed additive on broiler performance and carcass quality. *The Journal of Applied Poultry Research*, 17: 202–210.

**Burnell, T.W., Cromwell, G.L. and Stahly, T.S. (1988).** Effects of dried whey and copper sulfate on the growth responses to organic acid in diets for weanling pigs. *Journal of Animal Science* ,66(5):1100-1108.

**Byarugaba, A.K. (2010).** Mechanism of antimicrobial resistance. In *Antimicrobial Resistance in Developing Countries* (Eds.) A. de J.Sosa, D.K. Byarugaba, C. Amabile, P-R. Hsueh, S. Kariuk, I. N. Okeke). Springer, NY. pp 15-26.<http://www.springer.com/978-0-387-89369-3> Retrieved 10<sup>th</sup> January, 2014.

**Castro, M. and Elias, A. (1978).** Effect of inclusion of Zeolite in final molasses- based diets of performance of growing and fattening pigs. *Cuban Journal of Agricultural Science*, 12:69-75.

**Catry, B, Laevens, H, Devriese, L.A, Opsomer, G. and De Kruif, A. (2003).** Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology and Therapeutics*, 26: 81-93.

**Chiang, S.H. and Hsieh, W.M. (1995).** Effect of direct fed microorganisms on broiler growth performance and litter ammonia level. *Asian-Australasian Journal of Animal Science*, 8:159-162.

**Cromwell, G.L. (2001).** Antimicrobial and Promicrobial agents in Swine Nutrition, 2<sup>nd</sup> edition. ( Ed: A. J. Lewis and L. L. Southern, ed. CRC Press) Boca Raton, Florida. 401pp.

**Cunha, T. J., Burnside, J.E., Meadolis, G.B., Edwards, H.M., Benson, R.H., Pearson, A.M. and Glasscock, R.S. (1950).** Effect of AFP Supplement on efficiency of feed utilization for the pig. *Journal of Animal Science* 9:615-618.

**Davis, M.E., Parrott., T, Brown, D.C., Rodas., B. Z. De., Johnson, Z.B., Maxwell., C.V. and Rehberger, T. (2008).** Effect of a *Bacillus*- based direct-fed microbial feed supplement on growth performance and pen cleaning characteristics of growing- finishing pigs. *Journal of Animal Science*, 86:1459-1467.

**De Los Santos, J.R.G, Storch, O.B. and Gil-Turnes, C. (2005).** *B. cereus* Var.toyoii and *Saccharomyces boulardii* increased feed efficiency in broilers infected with *Salmonella enteritidis*. *British Poultry Science*. 46(4): 494- 49.

**Dei, H. K. Oware-Appiah, E. and Bawah, J. (2010).** The efficacy of Rumen 3 enhancer as a feed additive for broiler chickens. Proceedings of the 30<sup>th</sup> Biennial Conference of Ghana Animal Science Association, ( GASA). 208-213. August 26<sup>th</sup>-27<sup>th</sup>, Animal Research Institute, Accra.

**Dibner, J.J. and Buttin, P. (2002).** Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism *Journal of Applied Poultry Research*, 11: 453-463.

**Dibner, J. J. and Richards, J. D. (2005).** Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Science*, 84:634-643.

**Diebold, G. and Eidelsburger, U. (2006).** Acidification of diets as an alternative to antibiotic growth promoters. In: ( Eds: D Barug, de Jong, Ak Kies, M.W.A . Verstegen.)

Antimicrobial Growth Promoters. Wageningen Academic Publishers, The Netherlands. pp. 311-327.

**Dominy, N.J., Davoust, E. and Minekus, M. (2004).** Adaptive function of soil consumption an in vitro study modeling the human stomach and small intestine. *The Journal of Experimental Biology*, 207; 319-324.

**Doyle, M.E. (2001).** Alternatives to antibiotic use for growth promotion in animal husbandry. Food Research Institute, University of Wisconsin. Madison. pp 1-17.

**Džidic, S., Šušković, J. and Blazenka, K. (2008).** Antibiotic resistance mechanisms in bacteria: Biochemical and genetic aspects. *Food Technology and Biotechnology*, 46(1) 11-21.

**Eckel, B., Kirchgessner, M. and Roth, F.X. (1992).** Influence of formic acid on daily weight gain, feed intake, feed conversion rate and digestibility. *Journal of Animal Physiology and Animal Nutrition* 67 (2): 93-100.

**Edens, F. W., Parkhurst, C. R., Casas, I. A. and Dobrogosz, W. J. (1997).** Principles of ex ovo competitive exclusion and in ovo administration of *Lactobacillus reuteri*. *Poultry Science*, 76(1): 179-196.

**European Food Safety Authority (EFSA). (2003).** Feed additives-breaking legislation in European Union. *Veterinary Science Tomorrow*, 23: 81-94.

**FAO/WHO (2002).** Guidelines for the evaluation of probiotics in food. Published online at [http://fao.org/es/esn/food/wg\\_report2.pdf](http://fao.org/es/esn/food/wg_report2.pdf) Accessed on 12/10/2013.

**Food and Drug Administration (FDA) (1998).** Approved Animal Drug List . Drug Information Lab., College of Vet. Medicine, Blacksburg, Virginia. Vol. XII, NO. II.

**Food and Drug Administration (FDA). (2013).** New Animal Drugs and New Animal Drug Combination Products, Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209. Guidance for Industry (# 213), pp 1-18.

**Friend, B.A. and Shahani, K.M. (1984).** Nutritional and therapeutic aspects of *Lactobacilli*. *Journal of Applied Nutrition*, 36:125-53.

**Fuller, R. (1989).** Probiotics in man and animals. *Journal of Applied Bacteriology*, 66: 365-378.

**Fuller, R. (1995).** Probiotics, their development and use. In: Old Herborn University Seminar Monograph 8. (Eds van der Waaji, D., Heidt, P. J. and Rusch, V. C.). Institute for Microbiology and Biochemistry, Herborn-Dill. pp 1-5.

**Galan, E. (1996).** Properties and applications of palygorskite- sepiolite clays. *Clay Minerals*, 31: 443-453.

**Ganeshkumar, S., Gnanaraj, P. T., Kumar, T. S., Karthickeyan, S.M.K. and Murugan, M. (2009).** Effect of probiotic supplementation on the carcass traits and sensory qualities of swill fed pork. Tamilnaduj. *Veterinary and Animal Sciences*, 5(4): 157-160.

- Garrett, R.H. and Grisham, C. M. (1999).** Biochemistry 2<sup>nd</sup> edition, Saunders college publishing London. 131pp.
- Genstat Statistical Package (2009).** Genstat 12<sup>th</sup> Edition, Version 7.2 VSN International Limited, UK.
- Giang, H.H., Viet, T.Q., Ogle., B. and Lindberg, J.E. (2010a).** Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livestock Science*, 129: 95-103.
- Giang, H.H., Viet, T.Q., Ogle., B. and Lindberg, J.E. (2010b).** Effects of different probiotic complexes of lactic acid bacteria on growth performance and gut environment of weaned piglets. *Livestock Science*, 133:182-184.
- Gibson, G.R and Roberfroid, M.B. (1995).** Dietary modulation of the human colonic microbiota: introducing the concept of Prebiotics. *Journal of Nutrition* , 125: 1401-1412.
- Gibson, G.R. (2004).** From probiotics to prebiotics and a healthy digestive system. *Journal of Food Science*, 69: 141-143.
- Giesting, D.W. and Easter, R.A. (1991).** Effect of protein source and fumaric acid supplementation on apparent ileal digestibility of nutrients by young pigs. *Journal of Animal Science* 69,:2497-2503.
- Gillespie, R. J. (1998).** Modern Livestock and Poultry Production (4th ed). Delmar Pub. Inc. pp 181-247.
- Gracey, J. F., Collins, D.S. and Martins, R. J. (1999).** Meat Hygiene (10<sup>th</sup> Edition). Publishers W. B. Saunders Company Ltd. pp 299-320.
- Gratz, S.W., Mykkannen, H. and El-Nezami, H.S. (2010).** Probiotics and gut health: a special focus on liver diseases. *World Journal of Gastroenterology*, 16(4): 403.
- Greenwood, D. and Whitley, R. (1997).** Modes of action. Antibiotics and Chemotherapy, 7th ed. Churchill Livingstone, New York, NY, pp 10-22.
- Guarner, F. and Schaafsma, G. J. (1998).** Probiotics. *International Journal of Food Microbiology*, 39(3), 237-238.
- Gwendolyn J. (2010).** Feeding DFMs offers piglets a healthy start. *Feedstuffs*, 82 (44):1-3
- Hale, O.M. and Newton, G.L. (1979).** Effects of a nonviable *Lactobacillus* species fermentation product on performance of pigs. *Journal of Animal Science*, 48(4): 770-775.
- Hardy, B. (2002).** The issue of antibiotics use in the livestock industry: what have we learned? *Animal Biotechnology*, 13:129-147.
- Helbig, J. (2001).** Biological control of Botrytis Cinerea pers. Ex fr. In straw berry by paenibacillus polymyxa (isolate 18191). *Journal of Phytopathology*. 144: 265-273.

**Herias, M.V., Hesse, C., Telemo, E., Midtvedt, T., Hanson, L.A. and Wold, A.E. (1999).** Immunomodulatory effects of *Lactobacillus plantarum* colonizing the intestine of gnotobiotic rats. *Clinical and Experimental Immunology*, 116:283-290.

**Hong, J.W., Kim, I.H., Kwon, O.S., Kim, J.H., Min, J.B. and Lee, W.B. (2002).** Effects of dietary probiotics supplementation on growth performance and fecal gas emission in nursing and finishing pigs. *Journal of Animal Science and Technology*, 44: 305-314.

**Hooper, L. V., Wong, M. H., Thelin, A., Hansson, L., Falk, P. G. and Gordon, G. I. (2001).** Molecular analysis of commensal host-microbial relationship in the intestine. *Science*, 291: 881-884.

**Houdijk, J.G.M. (1998).** Effects of Non-Digestible Oligosaccharides in Young Pig Diets, PhD Thesis: Wageningen University, Netherlands 1998.141pp.

**Huber, J.T. (1990).** The Fungal and yeast culture story in Lactating dairy cows. Proceedings of South West Nutrition Management Conference Tempe City Arizona. pp.87-94. January 15<sup>th</sup> - 17<sup>th</sup>.

**Hume, M.E., Kubena, L.F., Edrington, J.S., Donskey, C.J., Moore, R.W., Ricke, S.C. and Nisbet, D.J. (2003).** Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. *Poultry Science*, 82: 1100-1107.

**Hutjens, M. F. (1991).** Feed additives Veterinary Clinics North America: Food Animal Practice. 7:2:525.

**Hutjens, M.F. (2007).** Feed management: A Key Ingredient in Livestock and Poultry Nutrient Management. Direct Fed Microbial Products (DFM) pp 1-5.

**Isley, S.E, Miller, H.M, Greathead, H.M.R. and Kamel, C. (2003).** Plant extracts as supplements for lactating sows: effects on piglet performance, sow food intake and diet digestibility. *Animal Science*, 77:247-254.

**Isley, S.E. and Miller, H.M. (2005).** Effects of dietary Quillaja Saponin and Curcumin on the performance and immune status of weaned piglets. *Journal of Animal Science* 83: 82-88.

**Isolauri, E., Sütas, Y., Kankaanpää, P., Arvilommi, H. and Salminen, S. (2001).** Probiotics effects on immunity. *The American Journal of Clinical Nutrition*, 73(2): 444s-450s.

**Iwamatsu, S, and Sawada T. (1988).** Relationship between serotypes, dermonecrotic toxin production of *Pasteurella multocida* isolates and pneumonic lesions of porcine *Veterinary Science*, 50:1200-1206.

**Jacela, J. Y., DeRouchey, J. M., Tokach, M. D., Goodband, R. D., Nelssen, J. L., Renter, D. G. and Dritz, S. S. (2010).** Feed additives for swine: Fact sheets – flavors and mold inhibitors, mycotoxin binders, and antioxidants. *Journal of Swine Health and Production*, 18 (1):27–32.

**Jasek, S.R., Kalinowska, R., Knecht, D. and Pawiak, R. (1992).** Effect of Biogen probiotic addition on reproduction results and physiological indices in pigs. 2. Effect of Biogen- T dietary additive on results of rearing weaners. *Rocznik Naukowych Zootechnicki*, 31: 227-229.

- Jin, L.Z.Y.W, Ho, N.A. and Jalaludin, S. (1997).** Probiotics in Poultry: modes of action. *World's Poultry Science Journal*,53: 351-368.
- Jones, G. (2002).** Phytobiotics-a sustainable solution. *Feed Mix*, 10 (6):37-38.
- Jonsson, E. and Conway, P. (1992).** Probiotics for Pigs. In; Probiotics: The Scientific Basis, ( Ed. R. Fuller) Chapman and Hall, London, pp:260.
- Jost, M. and Bracher, J.A. (2000).** Yeast products as feed additives in weanling piglets. *Agrar Forschung*.7: 56-61.
- Kalavathy, R., Abdullahi, N., Jalaludin, S. and Ho, Y.W. (2003).** Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weights of organs of broiler chickens. *British Poultry Science*,44 (1), 139-144.
- Kamra, D.N. and Pathak, N.N. (1996).** Nutritional Microbiology of Farm Animals. Vikas Publishing House PVT Ltd. PP 168-169.
- Kellems, O.R and Church, D.C. (2002).** Livestock Feeds and Feeding (5th ed.). Prentice-Hall, New Jersey, pp 39-248.
- Khaksefidi, A. and Rahimi, S. (2005).** Effect of Probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. *Asian Australasian Journal of Animal Sciences*,18 (8),1153.
- Knarreborg, A., Miquel, N., Granli, T., and Jensen, B.B. (2002).** Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. *Animal Feed Science Technology*, 99 (1-4): 131-140.
- Kyriakis, S.C., Tsilyoiannis, V.K., Vlemmas, J., Sarkis, K., Tsinas, A., Alexopoulos, C. and Jansegers, L. (1999).** The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. *Research in Veterinary Science*, 67, 223-228.
- Lai, S. and Tabacchioni, S. (2009).** Ecology and biotechnological potential of paenibacillus polymyxa: a minireview. *Indian Journal of Microbiology*,49 (1):2-10.
- Lan, Y., Xun, S., Tamminga, S., Williams, B.A., Verstegen, M.W.A. and Erdi, G. (2004).** Real- time PCR detection of lactic acid bacteria in cecal contents of Eimeria tenella- infected broilers fed soybean oligosaccharides and soluble soybean polysaccharides. *Poultry Science*, 83:1696-1702.
- Lantei, N. (2008).** The effects of Direct-Fed Microbials (DFM) and Mazorite (MAZ) on the growth performance and carcass characteristics of growing pigs. BSc. Thesis, KNUST, Kumasi, 56 pp.
- Lee, S. J., Shin, N. H., Ok, J. U., Jung, H. S., Chu, G. M., Kim, J. D., Kim, I. H. and Lee, S. S. (2009).** Effects of dietary synbiotics from anaerobic microflora on growth performance, noxious gas emission and fecal pathogenic bacteria population in weaning pigs. *Asian-Australian Journal of Animal Science*, 22(8):1202-1208.

**Lessard, M. and Brisson, G.J. (1987).** Effect of a *Lactobacillus* fermentation product on growth, immune response and fecal enzyme activity in weaned pigs. *Canadian Journal of Animal Science*, 67:509.

**Lewis A.R. (2002).** CRC Dictionary of Agricultural Sciences. CRC Press. Boca Raton, 630.3 Ref Lew 012717. U.K. 675 pp.

**Liu, J.R., Lai, S.E. and Yu, B. (2007).** Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on a wheat- based diet. *British Poultry Science*, 48: 507- 514.

**Llosa, M., Gomis-Ruth, F. X., Coll, M. and de la Cruz, F. (2002).** Bacteria conjugation: a two-step mechanism for DNA transport. *Molecular Microbiology*, 45(1):1-8.

**Macpherson, A, J. and Harris, N.L. (2004).** Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology*, 4(6): 478-485.

**Marinho, M.C., Lordelo, M.M, Cunha., L.F. and Freire, J.P.B. (2007).** Microbial activity in the gut of piglets: I. Effect of prebiotic and probiotic supplementation. *Livestock Science*, 108: 236-239.

**Martinez, M., Castro, M., Hidalgo, K., Ayala, U., Perez, R., Hernandez, L. and Baez, L. (2004).** Effective utilization of natural zeolite for diarrhea control. *Cuban Journal of Agricultural Science*, 38(4) : 387-390.

**Matsuzaki, T. and Chin, J. (2000).** Modulating immune responses with probiotic bacteria. *Immunology and Cell Biology*, 78: pp 67-73.

**Matthew, A.G., Chattin, S.E., Robbins, C.M. and Golden, D.A. (1998).** Effects of a direct-fed yeast culture on enteric microbial populations, fermentations acids and performance of weanling pigs. *Journal of Animal Science*, 76:2138-2145.

**Matthew, A.G. (2003).** Development of antibiotic resistance in Livestock production. In: Proceedings of the 6<sup>th</sup> Minnesota Nutrition Conference. St Paul, Minnesota, pp 78-92.

**Matthew, A.G., Arnett, D.B., Cullen, P. and Ebner, P.D. (2003).** Characterization of resistance patterns and detection of apramycin resistance genes in *Escherichia Coli* isolated from swine exposed to various environmental conditions. *International Journal of Food Microbiology*, 89:11-20.

**Michigan State University, MSU (2011).** Molecular basis for antibiotic resistance. Available at [www.amrls.msu.edu/microbiology/molecular-basis](http://www.amrls.msu.edu/microbiology/molecular-basis) for antibiotics resistance/acquired resistance accessed on 30/10/12.

**Midilli, M., Alp, M., Kolabach, N., Muglah, O. H., Turan, N., Yilmaz, H. and Cakir, S. (2008).** Effects of dietary and probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. *South African Journal of Animal Science*, 38(1), 21-27.

**Morrow, J. (2002).** Swine Stress and Pathogen Shedding. Swine Welfare Fact Sheet, 1(4):1

**Mroz, Z. (2005).** Organic acids as potential alternatives to antibiotic growth promoters for pigs. *Advances in Pork Production*, 16:169-182.

**Murray, A.C. Hinton, A.J. and Buhr, R.J. (2007).** Effect of botanical probiotic containing *Lactobacillus* on growth performance and populations of bacteria in the cloaca and carcass rinse of broiler chickens. *International Journal of Poultry Science*, 5: 344-350.

**Narkeviciute, I., Rudzeviciene, O., Leviniene, G., Mociskiene, K., and Eidukevicius, R. (2002).** Management of Lithuanian children's acute diarrhoea with Gastrolit solution and dioctahedral smectite. *European Journal of Gastroenterology and Hepatology*, 14(4), 419-424.

**Nicola, B. (2010).** How DFM's work: Performance probiotics, Direct- Fed Microbials, pp 1-5. Available on line at <http://performanceprobiotics.com> Accessed on October 13<sup>th</sup> 2013.

**Nisbet, D.J. (2003).** Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. *Poultry Science* 82: 1100-1107.

**Okai, D. B., Olympio, O. S. and Anim, P. K., (2000).** Responses of grower-finisher pigs to diets containing varying levels of wheat bran with or without OPTIZYME – an exogenous enzyme complex. *Journal of the University of Science and Technology*, 20 (1/2/3): 54 – 61.

**Okai, D.B. and Bonsi, M.L.K. (1989).** Poultry and Pig Production Hand-Book for the Ghanaian Farmer. (ed. Okai, D.B, Olympio, O.S, Bonsi, M.L.K and Esi Sam) Degraft Graphics and Publishers. Kumasi 66pp.

**Okai, D.B., Boateng, M., Oppong-Anane, K. and Baah, J. (2010).** On-farm feeding trial of pigs using a Direct-Fed Microbial (DFM) known as Pig Performance Enhancer (RE-3). 1<sup>st</sup> Nigerian International Pig Summit, Institute of Agricultural Research and Training, Moore Plantation, Ibadan, Nigeria, Poster Presentation, November 2010.

**Osei D.Y., Wallace P.A., Amoah, K.O., Aseidu P. and Asafu-Adjaye A. (2013).** Effects of dietary supplementation with probiotic (RE3<sup>®</sup>) on reproductive performance of rabbits. Proceedings of the 18<sup>th</sup> Biennial Conference of the Ghana Society of Animal Production (GSAP), University of Ghana, Legon, Accra. 231-235. July 25<sup>th</sup>-26<sup>th</sup>.

**Pandey, R.M. and Upadhyay, S.K. (2012).** Food Additive. In: Food Additive, (Ed. Yehia El-Samragy), ISBN: 978-953-51-0067-6, In Tech, Available at: <http://www.intechopen.com/books/food-additive/food-additive> Accessed on the 10<sup>th</sup> February 2013.

**Papaoiannou, D. S., Kyriakis, C. S., Alexopoulos, C., Tzika, E. D., Polizopoulou, Z. S., and Kyriakis, S. C. (2004).** A field study on the effect of the dietary use of a clinoptilolite-rich tuff, alone or in combination with certain antimicrobials, on the health status and performance of weaned, growing and finishing pigs. *Research in Veterinary Science*, 76(1), 19-29.

**Papatsiros, V. G., Christodoulopoulos, G. and Filippopoulos, L. C. (2012).** The use of organic acids in monogastric animals (swine and rabbits). *Journal of Cell and Animal Biology*, 6(10): 154-159.

**Partanen, K. H. (2001).** Organic acids. Their efficacy and modes of action in pigs. In: Piva A, Bach Knudsen, K.E, Lindberg, J.E(Eds), Gut Environment of Pigs. Nottingham University Press, pp. 201-218.

**Partanen, K.H. and Mroz, Z. (1999).** Organic acids for performance enhancement in pig diets. *Nutritional Reviews*, 12:117-145.

**Pijoan, C., Lastra, A., Ramirez C. and Leman, A. D. (1984).** Isolation of toxigenic strains of *Pasteurella multocida* from lungs of pneumonic swine. *Journal of the American Veterinary Medicine Association*, 185:522-523.

**Playne, M. (2003).** Probiotics-how effective are they? *Microbiology Australia*, 24(1):1

**Pollman, D.S., Danielson, D.M. and Peo, E.R. (1980).** Effect of microbial feed additives on performance of starter and growing-finishing pigs. *Journal of Animal Science*, 51:577-581.

**Rahimi, M. (2009).** The effects of probiotic supplementation on the performance and humoral immune response of broiler chickens. Book of Proceedings, 2<sup>nd</sup> Mediterranean Summit of WPSA. Pp. 67-69. Antalya, Turkey. Oct 4-7<sup>th</sup>.

**Ramu, J., Clark, K., Woode, G. N., Sarr, A. B. and Phillips, T. D. (1997).** Adsorption of cholera and heat-labile *Escherichia coli* enterotoxins by various adsorbents: An in vitro study. *Journal of Food Protection*, 60(4), 358-362.

**Rao, S. O. (2007).** The effect of dietary supplementation of *Lactobacillus*-based probiotics on growth and gut environment of nursery pigs. M.Sc. Thesis submitted to the Graduate School of Texas Tech University, USA. 78 pp.

**Ravi, A.V., Musthafa, K.S., Jegathambal, G., Kathiresan, K. and Pandian, S.K. (2007).** Screening and evaluation of probiotics as a biocontrol agent against pathogenic vibrios in marine aquaculture. *Letters in Applied Microbiology*, 45 (2): 219-223.

**Rayes, N., Seehofer, D., and Neuhaus, P. (2009).** Prebiotics, probiotics, synbiotics in surgery- are they only trendy, truly effective or even dangerous? *Langenbecks Archives of Surgery*, 394 (3):547-555.

**Robert, K.M., Daryl, K.G., Mayes, P.A. and Rodwell, W. (2003).** Harper's Illustrated Biochemistry. International Edition. 26<sup>th</sup> Edition. pp. 1-693.

**Roberts, W.C. (2005).** Pericardial heart disease:its morphologic features and its causes, Baylor University Medical Center Proceedings,18 (1):38-55

**Rolfe, R. D. (2000).** The role of probiotics cultures in the control of gastrointestinal health. *Journal of Nutrition*, 130:396-402.

**Roselli, M., Finamore, A., Britti, M.S., Bosi, P., Oswald, I. and Megheri, E. (2005).** Alternatives to in-feed antibiotics in pigs: Evaluation of probiotics, zinc or organic acids as protective agents for the intestinal mucosa. A comparison of in vitro and in vivo results. *Animal Research*, 54:203-218.

- Ross, G.R., Gusils, C., Oliszewski, R., Holgado, S.C. and Gonzalez, S.N. (2010).** Effects of probiotic administration in swine. *Journal of Biosciences and Bioengineering*, 109: 545-549.
- Roura, E., Homedes, J. and Klasing, K. C. (1992).** Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. *Journal of Nutrition*, 122:2383–2390.
- Sanders, M. E. (2000).** Considerations for use of probiotic bacteria to modulate human health. *The Journal of Nutrition*, 130(2): 384S-390S.
- Santos, A. A., Ferket, P. R., Grimes, J. L. and Santos, F. B. O. (2005).** Reduction of intestinal *Salmonella spp.* colonization in turkeys by dietary wheat, triticale and enzyme supplementation. Southern Poultry Science Society 25th Annual Meeting, Atlanta, GA. Pp. 132-141.
- Santoso, O.U.K., Tanake, K., Ohtani, S. and Sakaida, M. (2001).** Effect of fermented product from *Bacillus subtilis* on feed conversion efficiency, lipid accumulation and ammonia production in broiler chicks. *Asian- Australian Journal of Animal Science*, 14: 333-337.
- Scantlebury- Manning, T. and Gibson, G.R. (2004).** Prebiotics. Best practice and research *Clinical Gastroenterology*, 18: 287-298.
- Scharek, L., Altherr, J.B., Toelke, C. and Schmidt, M.F. (2007).** Influence of the probiotic *Bacillus cereus* Var. *toyoi* on the intestinal immunity of piglets. *Veterinary Immunology and Immunopathology*, 120:136-147.
- Schleif, R. (1993).** Genetics and Molecular Biology (2<sup>nd</sup> Ed). The Johns Hopkins University Press, Baltimore. Pp. 227-264.
- Serna, A., Espinosa, E., Camacho, E.M. and Casadesus, J. (2010).** Regulation of Bacterial conjugation in microaerobiosis by host- encoded function arc AB and Sdh ABCD. *Genetics*, 184(4): 947-958.
- Shabani, R., Nosrati, M., Javandel, F., Alaw, G.A.A. and Kiourmars, H. (2012).** The Effect of probiotics on growth performance of broilers. *Annals of Biological Research*, 3(12) 5450-5452.
- Shin, M.S., Han, S.K., Ji, A., Kim, K.S. and Lee, W.K. (2008).** Isolation and characterization of bacteriocin- producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. *Journal of Applied Microbiology*, 105:2203-2212.
- Shirkey, T.W., Siggers, R.H., Goldade, B.G., Marshall, J.K., Drew, M.D., Laarveld, B. and Van Kessel, A.G. (2006).** Effects of Commensal bacteria on intestinal morphology and expression of proinflammatory cytokines in the gnotobiotic pig. *Experimental Biology and Medicine*, 231(18), 133-1345.
- Shu, Q., Qu, F. and Gill, HS. (2001).** Probiotic treatment using *bifidobacterium lactis* HNO19 reduces weaning diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model. *Journal of Pediatric Enterology Nutrition*, 33:171-177.

**Smits, C.H.M. (1996).** Viscosity of Dietary Fibre in Relation to Lipid Digestibility on Broiler Chicken, PhD Thesis; Wageningen University, Netherlands 1996. Pp 1-141.

**Soares, G. M. S., Figueiredo, L. C., Faveri, M., Cortelli, S. C., Duarte, P. M. and Feres, M. (2012).** Mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs. *Journal of Applied Oral Science*, 20(3):295-309.

**Starr, M.P. and Reynolds, D.M. (1951).** Streptomycin resistance of coliform *bacteria* from turkeys fed streptomycin. *American Journal of Public Health and the Nations Health* 41, no. 11 pt 1:1375-1380.

**Stephany, R. W. (2010).** Hormonal growth promoting agents in food producing animals. In: Doping in Sports. Hand Book of Experimental Pharmacology (Eds. Thieme, D and Hemmersbach P.). Springer-Verlag, Berlin Heidelberg. pp 355-367. Available at DOI 10.1007/978-3-540-79088-4\_16. Accessed on 15<sup>th</sup> /11/ 2013.

**Stokstad, E. L.R. and Jukes, T.H. (1950).** Further observations on the animal protein. *Experimental Biology and Medicine*, 73(3), 523-528.

**Szabo, I.L., Wieler, H., Tedin, K., Scharek-Tedin, L. and Taras, D. (2009).** Influence of a probiotic strain of *Enterococcus faecium* on *salmonella* enteric serovar typhimurium DT 104 infection in a porcine animal infection model. *Applied Environmental Microbiology*, 75:2621-2628.

**Takahashi, T., Nakagawa, E., Nara, T., Yajima, T. and Kuwata. T. (1998).** Effects of orally ingested *Bifidobacterium longum* on the mucosal IgA response of mice to dietary antigens. *Biology, Biotechnology and Biochemistry*, 62:10-15.

**Tenover, F. C. (2006).** Mechanism of antimicrobial resistance in bacteria. *The American Journal of Medicine*, 119(6A): S5-S10.

**Timmusk, S. and Wagner, E.G.H. (1999).** The plant- growth promoting rhizobacterium *paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. *Molecular plant- Microbe Interaction* 12: 951-959.

**Tsiloyiannis, V.K., Kyriakis, S.C., Vlemmas, J. and Sarris, K. (2001a).** The effect of organic acids on the control of porcine post-weaning diarrhoea. *Research in Veterinary Science* ,70:287-293.

**Tsiloyiannis, V.K., Kyriakis, S.C., Vlemmas, J. and Sarris, K. (2001b).** The effect of organic acids on the control of post-weaning oedema disease of piglets. *Research in Veterinary Science* ,70:281-285.

**Tung, C.M. and Pettigrew, J.E. (2006).** Critical review of acidifiers. National Pork Board. Pp 1-52. Available online at <http://www.pork.org/documents/pork> science. (Accessed on 10/04/ 13.)

**Verstegen, M.W. and Williams, B.A. (2002).** Alternatives to the use of antibiotics as growth promoters for monogastric animals. *Animal Biotechnology*. 13:113-127.

- Vitini, E., Alvarez, S., Medina, M., Medici, M., Budeguer, M. V. de. and Perdigon. G. (2000).** Gut mucosal immunostimulation by lactic acid bacteria. *Biocell: Official Journal of the Sociedades Latinoamericanas de Microscopia*, 24:223-232.
- Vondruskova, H., Slamova, R., Trckova, M., Zraly, Z. and Pavlik, I. (2010).** Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: A review. *Veterinarni Medicina*, 55 (5): 199–224.
- Wallace, P.A., Osei, D.Y., Aseidu, P., Amoah, K.O. and Asafu-Adjaye, A. (2012).** Influence of the probiotic, RE 3 on nutritional performance, haematological, immune status and carcass characteristics of rabbit reared under tropical conditions. *Online Journal of Animal and Feed Research*, 2 (5): 450-456.
- Wan, X. L., Yang, Z. B., Yang, W. R., Jiang, S. Z., Zhang, G. G., Johnston, S. L. and Chi, F. (2013).** Toxicity of increasing aflatoxin B1 concentrations from contaminated corn with or without clay adsorbent supplementation in ducklings. *Poultry Science*, 92:1244–1253. Available at <http://dx.doi.org/10.3382/ps.2012-02748> (Accessed on 1/09/13)
- Wang, Y., Cho, J.H., Chen, Y.J., Yoo, J.S., Huang, Y., Kim, H.J. and Kim, I.H. (2009).** The effect of probiotic Bioplus 2B® on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. *Livestock Science*, 120:35-42.
- Wenk, C. (2000).** Recent advances in animal feed additives such as metabolic modifier, antimicrobial agents, probiotics, enzymes and available minerals (Review). *Asian-Australian Journal Animal Science*, 13: 86-95.
- Wenk, C. (2003).** Herbs and botanicals as feed additive in monogastric animals. *Asian Australasian Journal of Animal Science* 16: 282-289.
- Wicklein, B., Darder, M., Aranda, P. and Ruiz-Hitzky, E. (2008).** Organically modified clays for uptake of mycotoxins. *Revista de la Sociedad Española de Mineralogia*,(1): 257-258
- Wikipedia (2014).** Probiotics. [en.wikipedia.org/wiki/probiotics](http://en.wikipedia.org/wiki/probiotics) Accessed on 31<sup>st</sup>/01/2014
- William, H.C. (2000).** Producing pigs without antibiotic growth promoters. *Advances in Pork Production* ,11:47-56.
- Witte, W. (1998).** Medical consequences of antibiotic use in agriculture. *Science*, 279: 996–997.
- Wu, X-Y. (2006).** Studies on the impact of probiotic bacteria on enteric microbial diversity and immune response. Ph. D. Thesis, School of Biological Sciences, University of Wollongong, New South Wales, Australia. 220pp. Available at: <http://ro.uow.edu.au/theses/596> (Accessed on 2/09/13)
- Zani, J.L., Da- Cruz, F.W., De Los Santos, A.F. and Gil-Turnes, C. (1998).** Effect of Probiotic CenBiot on the control of diarrhoea and feed efficiency in pigs. *Journal of Applied Microbiology*, 84:68-71.
- Zanu, H. K., Kagya-Agyemang, J. K., Kwenin, W. K. J., Bonsu, F. R. K., Antwi, E and Ateni, S. (2011).** Physiological responses of broiler chickens to neem (*Azadirachta indica*)

and Akakapenpen (*Rauvolfia vomitoria*) decoctions: performance and carcass characteristics. *International Journal of Poultry Science*, 10(9): 730-733.

**Zhao, G, Halbur, T. and Duane, C.P. (1995).** Colonization of oropharynx and nasal cavity of CDCD pigs by a nontoxigenic strain of *Pasteurella multocida* type D. *Swine Health and Production* 3(3):113-115.

**Zimmerman, D.R. (1986).** Role of sub-therapeutic antimicrobials in pig production. *Journal of Animal Science* 62(suppl. 3): 6-17.

**Zulkifli, I., Abdullah, N., Azrin, N.M. and Ho, Y.W. (2000).** Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. *British Poultry Science*, 41: 593-597.

KNUST



## 6.0.APPENDIX

### ANALYSIS OF VARIANCE (ANOVA)

#### GROWTH PERFORMANCE TRAITS (STARTER PHASE)

##### a. Initial weight, kg

Variate: INITIAL WEIGHT						
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3	0.000	0.000	0.00	1.000	2.033
Residual	16	36.800	2.300			
Total	19	36.800				

##### b. Daily feed intake, kg

Variate: DAILY FEED INTAKE						
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3	0.13179	0.04393	0.44	0.726	0.4224
Residual	16	1.58793	0.09925			
Total	19	1.71972				

##### c. Total feed intake, kg

Variate: TOTAL FEED INTAKE						
Source of variation	d.f.	s.s.	m.s	v.r	F pr.	LSD
TRT	3	336.0	112.0	0.77	0.526	16.14
Residual	16	2318.5	144.9			
Total	19	2654.5				

##### d. Daily weight gain, kg

Variate: DAILY WEIGHT GAIN						
Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.	LSD
TRT	3	0.02571	0.00857	0.28	0.836	0.2326
Residual	16	0.48146	0.03009			
Total	19	0.50717				

##### e. Total weight gain, kg

Variate: TOTAL WEIGHT GAIN						

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3	18.85	6.28	0.15	0.929	2.033
Residual	16	673.60	42.10			
Total	19	692.45				

f. Feed conversion efficiency

Variate: FCE						
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3	1.5832	0.5277	1.12	0.369	0.919
Residual	16	7.5100	0.4694			
Total	19	9.0932				

g. Final weight, kg

Variate: final weight						
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3	18.85	6.28	0.10	0.956	10.39
Residual	16	961.70	60.11			
Total	19	980.15				

## ANALYSIS OF VARIANCE (ANOVA)

### GROWTH PERFORMANCE TRAITS (GROWER- FINISHER PHASE)

a. Initial weight, kg

Variate: initial weight						
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3	18.85	6.28	0.10	0.956	10.39
Residual	16	961.70	60.11			
Total	19	980.15				

b. Daily feed intake , kg

Variate: daily feed intake						
Source of variation	d.f (m.v.)	s.s.	m.s.	v.r	F pr.	LSD
TRT	3	0.07498	0.02499	0.79	0.519	0.2410
Residual	14 (2)	0.44205	0.03157			
Total	17 (2)	0.51347				

c. Total feed intake, kg

Variate: total feed intake						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r	F pr. LSD
TRT	3		728.9	243.0	0.28	0.842 40.26
Residual	14	(2)	12331.8	880.8		
Total	17	(2)	12992.4			

d. Daily weight gain, kg

Variate: daily weight gain						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r	F pr. Lsd
TRT	3		1.7764	0.5921	2.27	0.125 0.692
Residual	14	(2)	3.6477	0.2606		
Total	17	(2)	5.2807			

e. Total weight gain, kg

Variate: total weight gain						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r	F pr. LSD
TRT	3		9.76	3.25	0.05	0.983 10.56
Residual	14	(2)	848.54	60.61		
Total	17	(2)	856.50			

f. Feed conversion efficiency

Variate: FCE						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r	F pr. LSD
TRT	3		0.34884	0.11628	1.74	0.204 0.3052
Residual	14	(2)	0.93306	0.06665		
Total	17	(2)	1.23824			

g. Final weight, kg

Variate: final body weight						
Source of variation	d.f.		s.s.	m.s.	v.r.	F pr. LSD
TRT	3		6.009	2.003	0.65	0.599 2.389
Residual	14		43.438	3.103		
Total	17		48.736			

## ANALYSIS OF VARIANCE (ANOVA)

### GROWTH PERFORMANCE TRAITS (OVERALL PERFORMANCE)

a. Daily feed intake, kg

Variate: daily feed intake							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		0.04220	0.01407	0.57	0.641	0.2122
Residual	14	(2)	0.34260	0.02447			
Total	17	(2)	0.38324				

b. Total feed intake, kg

Variate: total feed intake							
Source of variation	d.f.		s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		73.	24.	0.02	0.996	2.389
Residual	16		18479.	1155.			
Total	19		18552.				

c. Daily weight gain, kg

Variate: daily weight gain							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		0.019972	0.006657	1.04	0.404	0.1084
Residual	14	(2)	0.089367	0.006383			
Total	17	(2)	0.107403				

d. Total weight gain, kg

Variate: total weight gain							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		3.471	1.157	0.24	0.865	2.959
Residual	14	(2)	66.637	4.760			
Total	17	(2)	69.736				

e. Feed conversion efficiency

Variate: feed conversion efficiency						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. LSD
TRT	3		0.31380	0.10460	1.27	0.323 0.3894
Residual	14	(2)	1.15371	0.08241		
Total	17	(2)	1.41801			

f. Duration

Variate: duration						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. LSD
TRT	3		687.2	229.1	0.54	0.660 27.83
Residual	14	(2)	5894.7	421.1		
Total	17	(2)	6522.4			

g. Final body weight, kg

Variate: final body weight						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. LSD
TRT	3		6.009	2.003	0.65	0.599 2.389
Residual	14	(2)	43.438	3.103		
Total	17	(2)	48.736			

ANALYSIS OF VARIANCE (ANOVA)

MICROBIAL PROFILE OF GROWER-FINISHER PIGS.

Variate: TOT CNT						
Source of variation	d.f.	s.s.	m.s.	v.r .	F pr.	LSD
TRT	3	7.3927	2.4642	2.55	0.092	1.318
Residual	16	15.4533	0.9658			
Total	19	22.8460				

ANALYSIS OF VARIANCE (ANOVA)

CARCASS CHARACTERISTICS

a. Backfat, cm

Variate: BACKFAT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.9730	0.3243	0.46	0.713 1.136
Residual	14	(2)	9.8121	0.7009		
Total	17	(2)	10.6982			

b. Belly, kg

Variate: BELLY						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.2741	0.0914	0.58	0.635 0.5364
Residual	14	(2)	2.1889	0.1563		
Total	17	(2)	2.4344			

c. Carcass length, kg

Variate: CAR LTH						
Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3	202.70	67.57	1.84	0.186	8.62
Residual	14 (2)	513.85	36.70			
Total	17 (2)	689.54				

d. Empty GIT, kg

Variate: EMPTY GIT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.12331	0.04110	0.44	0.725 0.4124
Residual	14	(2)	1.29375	0.09241		
Total	17	(2)	1.40125			

e. Fillet, kg

Variate: FILLET						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.009375	0.003125	1.09	0.384 0.0725
Residual	14	(2)	0.040000	0.002857		
Total	17	(2)	0.047778			

f. Full GIT, kg

Variate: FULL GIT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		1.6125	0.5375	1.16	0.361 0.0924
Residual	14	(2)	6.5000	0.4643		
Total	17	(2)	8.0757			

g. Head, kg

Variate: HEAD						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.9185	0.3062	2.68	0.087 0.4581
Residual	14	(2)	1.5964	0.1140		
Total	17	(2)	2.3662			

h. Heart, kg

Variate: HEART						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.00553	0.001844	1.24	0.331 0.05222
Residual	14	(2)	0.020750	0.001482		
Total	17	(2)	0.025694			

i. Kidneys, kg

Variate: KIDNEYS						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.001023	0.000341	0.10	0.958 0.0785
Residual	14	(2)	0.046875	0.003348		
Total	17	(2)	0.047778			

j. LEAF FAT, KG

Variate: LEAF FAT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.01534	0.00511	0.28	0.840 0.1836
Residual	14	(2)	0.25638	0.01831		
Total	17	(2)	0.27069			

k. Liver, kg

Variate: LIVER						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.560461	0.186820	22.38	<.001 0.1239
Residual	14	(2)	0.116875	0.008348		
Total	17	(2)	0.572778			

l. Loin, kg

Variate: LOIN						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		5.7070	1.9023	5.50	0.010 0.798
Residual	14	(2)	4.8409	0.3458		
Total	17	(2)	9.6163			

m. P2 measurement, kg

Variate: P2 MEASUREMENT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.02459	0.00820	0.61	0.622 0.1578
Residual	14	(2)	0.18950	0.01354		
Total	17	(2)	0.21111			

n. Respiratory tract, kg

Variate: RESPIRATORY TRACT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.03194	0.01065	0.26	0.851 0.2732
Residual	14	(2)	0.56775	0.04055		
Total	17	(2)	0.59625			

o. Shoulder, kg

Variate: SHOULDER						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.14596	0.04865	1.16	0.361 0.2782
Residual	14	(2)	0.58888	0.04206		
Total	17	(2)	0.70903			

p. Spleen, kg

Variate: SPLEEN						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.000125	0.000042	0.03	0.993 0.05254
Residual	14	(2)	0.021000	0.001500		
Total	17	(2)	0.021111			

q. Empty stomach, kg

Variate: EMPTY STOMACH						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.14638	0.04879	1.71	0.211 0.2293
Residual	14	(2)	0.40000	0.02857		
Total	17	(2)	0.54236			

r. Thigh, kg

Variate: THIGH							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		0.6405	0.2135	1.64	0.226	0.4897
Residual	14	(2)	1.8249	0.1303			
Total	17	(2)	2.3711				

s. Trotters, kg

Variate: TROTTERS							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		0.02140	0.00713	0.40	0.758	
0.1819							
Residual	14	(2)	0.25187	0.01799			
Total	17	(2)	0.27111				

t. Viscera, kg

Variate: VISCERA							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		1.8500	0.6167	0.84	0.493	1.610
Residual	14	(2)	10.2388	0.7313			
Total	17	(2)	11.9263				

u. Warm weight with head, kg

Variate: WARM with head							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	LSD
TRT	3		5.092	1.697	1.46	0.268	1.463
Residual	14	(2)	16.287	1.163			
Total	17	(2)	20.691				

v. Warm weight without head, kg

Variate: WARM_without_head						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. LSD
TRT	3		17.528	5.843	2.78	0.080 1.965
Residual	14	(2)	29.375	2.098		
Total	17	(2)	44.611			

w. Chilled weight, kg

Variate: CHILLED WEIGHT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. LSD
TRT	3		3.3898	1.1299	1.14	0.369 1.353
Residual	14	(2)	13.9375	0.9955		
Total	17	(2)	17.0000			

x. Loin eye area, cm<sup>2</sup>

Variate: LOIN EYE AREA						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. LSD
TRT	3		71.00	23.67	0.86	0.486 7.130
Residual	14	(2)	386.81	27.63		
Total	17	(2)	448.45			

y. Chilled dressed weight, %

Variate: CHILLED DRESSED WEIGHT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		1.419	0.473	0.19	0.900 2.125
Residual	14	(2)	34.367	2.455		
Total	17	(2)	35.720			

z. Predicted lean (%)

Variate: predicted_lean						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		25.62	8.54	0.72	0.558
Residual	13	(3)	154.30	11.87		
Total	16	(3)	177.76			

aa. Dressing percentages with head (%)

Variate: dressing%_with_head						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		1.271	0.424	0.12	0.949 2.591
Residual	14	(2)	51.092	3.649		
Total	17	(2)	52.290			

bb. Dressing percentages without head (%)

Variate: dressing%_no_head						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		18.544	6.181	3.11	0.061 1.914
Residual	14	(2)	27.868	1.991		
Total	17	(2)	44.033			

### ANALYSIS OF VARIANCE (ANOVA)

#### CARCASS CHARACTERISTICS (RELATIVE)

a. Belly, kg

Variate: BELLY						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.7931	0.2644	0.79	0.521 0.7865
Residual	14	(2)	4.7059	0.3361		
Total	17	(2)	5.4134			

b. Empty stomach, kg

Variate: EMPTY STOMACH							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		0.30969	0.10323	1.92	0.172	0.3141
Residual	14	(2)	0.75086	0.05363			
Total	17	(2)	1.05096				

c. Empty GIT, kg

Variate: EMPTY GIT							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		0.1694	0.0565	0.35	0.789	0.5443
Residual	14	(2)	2.2544	0.1610			
Total	17	(2)	2.3965				

d. Fillet, kg

Variate: FILLET							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		0.017825	0.005942	1.12	0.375	0.0988
Residual	14	(2)	0.074332	0.005309			
Total	17	(2)	0.088943				

e. FULL GIT, KG

Variate: FULL GIT							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		3.3421	1.1140	1.49	0.260	1.1721
Residual	14	(2)	10.4526	0.7466			
Total	17	(2)	13.6608				

f. Head, kg

Variate: HEAD							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		1.2435	0.4145	2.67	0.088	0.5345
Residual	14	(2)	2.1736	0.1553			
Total	17	(2)	3.2048				

g. Heart, kg

Variate: HEART						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r. F pr.	L.S.D
TRT	3		0.009329	0.003110	1.31 0.309	0.0660
Residual	14	(2)	0.033151	0.002368		
Total	17	(2)	0.041509			

h. Kidneys, kg

Variate: KIDNEYS						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r. F pr.	L.S.D
TRT	3		0.001628	0.000543	0.09 0.966	0.1075
Residual	14	(2)	0.087952	0.006282		
Total	17	(2)	0.089428			

i. Leaf fat, kg

Variate: LEAF FAT						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.F pr.	L.S.D
TRT	3		0.03017	0.01006	0.27 0.849	0.2638
Residual	14	(2)	0.52964	0.03783		
Total	17	(2)	0.55777			

j. Liver, kg

Variate: LIVER						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r. F pr.	L.S.D
TRT	3		1.09131	0.36377	24.71 <.001	0.1646
Residual	14	(2)	0.20608	0.01472		
Total	17	(2)	1.08942			

k. Loin, kg

Variate: LOIN						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r. F pr.	L.S.D

TRT	3		11.1266	3.7089	4.91	0.015	1.1785
Residual	14	(2)	10.5677	0.7548			
Total	17	(2)	19.9841				

l. Respiratory tract, kg

Variate: RESPIRATORY TRACT							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		0.08261	0.02754	0.42	0.739	0.3455
Residual	14	(2)	0.90837	0.06488			
Total	17	(2)	0.98238				

m. Shoulder, kg

Variate: SHOULDER							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		0.32510	0.10837	1.27	0.322	0.3960
Residual	14	(2)	1.19286	0.08520			
Total	17	(2)	1.46869				

n. Spleen, kg

Variate: SPLEEN							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		0.000123	0.000041	0.02	0.997	0.0703
Residual	14	(2)	0.037591	0.002685			
Total	17	(2)	0.037700				

o. Thigh, kg

Variate: THIGH							
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.	L.S.D
TRT	3		1.4587	0.4862	1.89	0.178	0.6879
Residual	14	(2)	3.5999	0.2571			
Total	17	(2)	4.8418				

p. Trotters, kg

Variate: TROTTERS						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		0.02744	0.00915	0.29	0.833 0.2416
Residual	14	(2)	0.44395	0.03171		
Total	17	(2)	0.46914			

q. Viscera, kg

KNUST

Variate: VISCERA						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr. L.S.D
TRT	3		4.4653	1.4884	1.65	0.223 1.2889
Residual	14	(2)	12.6407	0.9029		
Total	17	(2)	16.6216			

