GROWTH AND REPRODUCTIVE PERFORMANCE AND MATERNAL AND PIGLET BLOOD PROFILES OF PIGS FED DIETS CONTAINING DIFFERING DIRECT-FED MICROBIAL PRODUCTS

BY

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DECLARATION

I, **Yaw Oppong Frimpong**, hereby declare that the work presented in this thesis is the result of my own effort and no such previous application for a degree in this University or elsewhere has the same work being presented.

All sources of information have been acknowledged by reference to authors.

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ABSTRACT

A study was carried out to ascertain the effects of three DFM products (RE3™, RE3 PLUS and a combination of RE3™ and P3) on the growth performance, gut microflora, blood profile and reproductive performance of gilts and on growth performance and blood profile of their piglets. Sixteen Large White gilts of an average initial weight of 41.66kg were randomly allocated to 4 dietary treatments (T1- Control, basal diet without DFM, T2- 1ml RE3™ per kg feed, T3- 1ml RE3 PLUS per kg feed and T4- 1ml RE3™ + 0.5ml P3 per kg feed) in a completely randomized design. The experiment consisted of 2 phases, i.e. a grower-finisher and a gestation and lactation phases for the gilts. Diets containing 23, 18 and 16% CP were prepared and offered to piglets, growing and lactating gilts and pregnant gilts, respectively during the entire experiment. Growing-finishing gilts were allowed 4% of body weight of feed until they attained a body weight of 80±0.5kg before the feed allowance was reduced to 3% of body weight. Pregnant gilts were offered a daily feed allowance of 2kg while lactating gilts were provided 5kg of feed. Piglets were offered *ad libitum* access to creep feed at 2 weeks of age. Water was provided *ad libitum*. Weighing was done weekly for gilts until they became pregnant whilst piglets were weighed within 24 hour after they were farrowed and subsequently weekly until weaning at 4 weeks of age. Blood samples were taken from gilts at the start of the experiment and after the first phase of the experiment (Week 12) for haematology, serum biochemistry and immunological studies. Blood samples were taken from piglets within 24 hours after birth and after every week until they were weaned. Faecal samples were also taken from gilts for microbial analysis after the 1st phase of the experiment. The study spanned a period of 32 weeks. It was concluded that with the exception of blood serum protein, the 3 different probiotic products did not seem to significantly influence growth performance, reproductive performance, blood profile

and gut microflora of the gilts, and the growth performance of piglets. However, DFM supplementation resulted in decreased concentrations of RBC and MCHC but increased concentrations of monocytes, PLT and IgM in piglets.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	DESCRIPTION
AAFCO	Association of American Feed Control Officials
AGP	Antimicrobial Growth Promoters
AHGP	Antibiotic and Hormonal Growth Promoters
CFU	Colony Forming Unit
CP	Crude Protein
DES	Diethylstilboestrol
DFM	Direct Fed Microbials
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked-Immunosorbent Serologic Assay
FDA	Food and Drug Administration
GH	Growth Hormone
HB	Haemoglobin
HCT	Haematocrit
HGP	Hormonal Growth Promoters
HSCAS	Hydrated Sodium Calcium Aluminosilicates
LAB	Lactic Acid Bacteria
MCHC	Mean Cell Haemoglobin Concentration
MCH	Mean Cell Haemoglobin
MCV	Mean Cell Volume
MGA	Melengestrol Acetate

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NSP	Non-Starch Polysaccharides
PBP	Penicillin Binding Proteins
PCDD	Polychlorinated Dibenzodioxins
PLT	Platelet
RBC	Red Blood Cells
RNA	Ribonucleic Acid
SCFA	Short Chain Fatty Acids
TRH	Thyrotrophin-Releasing Hormone
VFA	Volatile Fatty Acids
WBC	White Blood Cells

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DEDICATION

I dedicate this thesis to my late mum, Ms. Comfort A. Antwi.

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CHAPTER 1

1.0 INTRODUCTION

The domestication of farm animals granted man endless access to animal protein and other animal products. Because of increases in human population which bring about increase in demand for animal products, among other things, the need arose to increase the population density of farm animals with its resultant effects like decreased floor spacing, increased stress, increased incidence of pathogens and also the increase in susceptibility of farm animals to these pathogens (Cook, 2004). Also, the competition between man, farm animals and industries for some common food ingredients has called for the use of inferior feed ingredients in animal production, predisposing animals to diseases caused by some of the pathogens in the feed.

Conditions associated with pregnancy and maternal tolerance of fetuses also call for a compromise in the immune system of the dam (Abdul-Rahman *et al*., 2011; Sanz, 2011) leading to the susceptibility of the dam to pathogenic bacterial and viral diseases which, in the end, may harm not only the mother but also the fetus. Furthermore, the introduction of piglets to solid feed, according to Lesniewska *et al. (*2000) and Morrow (2002) may constitute stress since it leads to several changes within the animal. These changes may include struggling of piglets for feed as well as changes in the morphology of the intestines. Lesniewska *et al.* (2000) for example, observed changes in the motility pattern of the small intestines when piglets were fed solid diets.

Hence, in the late 1950's, according to Buchanan *et al.* (2008), Cook (2004) and Holtz (2009), hormones and sub-therapeutic levels of antibiotics were allowed in animal feeds to help reduce the levels of infestation of some pathogenic bacteria, reduce mortality due to compromised

sanitary and feeding conditions and also to promote growth of animals. The use of hormones and low doses of antibiotics in animal feed gained root especially in the developed world such that as at 1997, farming was the second largest consumer of antibiotics in Europe (Hong *et al.*, 2004).

With time, it was observed that some of the pathogenic microbes in humans were becoming resistant to some of these antibiotics and various scholars, according to Joerger (2003) and Witte (1998), attributed the situation to the possibility that some antibiotic residues are passed on from farm animals to man in the form of meat, milk; and manure which is applied onto crops in most organic farms, etc. Also, it was observed that hormone use in animals was associated with cancers, impotence, autoimmune diseases, etc. (Stephany, 2010). This led to some countries banning the use of some antibiotics and hormones. The UK for example, banned the use of penicillin and tetracycline as growth promoters in 1970, Sweden and Denmark banned the use of all growth promoting antibiotics in 1986 and 1999, respectively and the USA banned the use of enrofloxacin in 2005 (Buchanan *et al.*, 2008). It is worth mentioning that, all hormones have been banned in the EU and only 7 hormones are allowed in the US and Canada (Holtz, 2009) for animal feeding. The success of these measures in the above stated countries motivated the EU to ban the sub-therapeutic use of all antibiotics in 2006 (Vondruskova *et al.*, 2010).

According to Cook (2004), because of the high demand for animal products and the need to cut cost, there was the need to find some natural alternatives to antibiotic and hormonal growth promoters (AHGP) which were safer and healthier to humans as well as sustainable because the EU's move may steer a worldwide fight against all AHGP's.

Numerous strategies cropped up whilst some old ones also gained renewed attention. Some of the possible alternatives under research include dietary enzymes (Cook, 2004), organic acids,

prebiotics, phytobiotic, probiotics/ Direct Fed Microbials (DFM) (Abdul-Rahman *et al.*, 2011; Buchanan *et al.*, 2008 and Rahimi, 2009), bacteriocins, antimicrobial peptides, bacteriophages (Joerger, 2003) and nutraceuticals (Denariaz *et al.*, 1999).

For some time now, probiotics, which are viable, friendly bacterial or fungal cultures have been found to be able to improve the balance of intestinal flora and exert beneficial effects on the individual in which it is being administered (Benno and Mitsuoka, 1992; Rolfe, 2000). Their use has gained popularity due probably to the numerous beneficial effects they offer the animal. Denariaz *et al*. (1999) and Sarkar (2011) stated that, these beneficial microbes perform several function like allergy prevention, synthesis and the enhancement in the bioavailability of nutrients, induction of hypocholesterolemic effects, improvement in digestion, improvement of the immune system, prevention of cancers, growth enhancement and well-being of animals.

In Ghana and Africa as a whole, there is a dearth of research on the effects of probiotics on different production indices of farm animals; some have shown significant improvement in animal growth and production (Okai *et al.*, 2010; Bonsu *et al.*, 2012) whilst most of the researches (Adusah, 2009; Brown, 2009; Amoah, 2010) have failed to indicate any clear cut headways. Moreover, most of the works done did not consider the effects of probiotic supplementation on immunology and reproductive performance of farm animals. It must also be mentioned that supplementation of DFM to animals is a dynamic part of animal nutrition and new products are introduced on regular basis; thus there is a need to study into the new products being introduced for animal production.

This study therefore sought to assess the effects of three DFM products produced and supplied by Basic Environmental Systems and Technology (BEST) Inc., Alberta, Canada (i.e. RE3™, RE3

PLUS and a combination of RE3™ and P3) on growth performance, economies of production, blood haematological, biochemical and immunological profile and the composition of microbes in the gut of growing-finishing and lactating gilts and on the growth performance and blood profile of their piglets/ progeny before weaning.

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1. Feed additives

Feed additives are compounds that may elicit response independent of the contribution of the animal's energy, amino acid, mineral and or vitamin requirement (Reese *et al.*, 2000). Lewis (2002) explained that feed additives may be added to a feed in order to improve its flavour, odour, appearance, extend the useful life and enhance natural properties of the feed and also to improve growth and health of the animal. Some feed additives are also added to correct deficiencies in the feed given to the animal. Feed additives are usually added to the diet of animals in small quantities (Gillespie, 1998; Kellems and Church, 2002).

2.1.1. Classification of feed additives

There are several ways of classifying feed additives, but a simple system of classification categorizes feed additives into two main groups based on whether the additive supplies nutrients to the animal or not (Banerjee, 1988). These two groups are described as nutritive and non-nutritive feed additives respectively.

1. Nutritive feed additives: This group consists of nutrient-containing substances and is normally added to balance the nutrient requirements of the animal which are not in their right or recommended quantities. Prominent additives under this group are minerals, vitamins and synthetic amino acids.

2. Non-nutritive feed additives: Additive which fall under this group do not supply animals with nutrients but rather may improve growth by improving feed conversion ratios,

preventing disease, detoxifying and preserving animal feed, improving feed intake, etc. Based on the European Food Safety Authority's system of classifying feed additives (EFSA, 2003), non-nutritive feed additives constitute the bulk of feed additives used in animal production and consists of the technological additives like pellet binders and organic acids which encourages easy handling and proper storage of feed; sensory additives which improves acceptability of feed by animals; coccidiostats and zootechnical additives which improve nutrient availability and as well as protecting animals from pathogenic infections.

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Years after their discovery and widespread use, some of the non-nutritive feed additives used in growth promotion such as antibiotics (Vondruskova *et al.,* 2010) and hormones (Stephany, 2010) have come under serious scrutiny due to the harmful health effects they pose to humans resulting in the need to find possible alternatives.

2.2. Growth promotion in farm animals

Human population growth, according to Cook (2004) increased the pressure on farmers and scientists to find possible ways of increasing yield per unit area in food production. This brought about researches into improving the genetic composition of both plants and animals to produce higher quantities of food in relatively shorter time periods. Also BCERF (2000) and Cook (2004) explained that strategies like intensive systems of animal farming with its resultant compromised floor spacing and high rates of pathogen shedding were also introduced to help produce more animal in small land holdings so as to satisfy the high demand for animal products. Furthermore, researches were done to find products that could help reduce waiting time and the amount of feed consumed by animals before slaughter, increase the quantity of milk produced by dairy animals and also curb the incidence of diseases associated with the high densities of pathogens in farms

(CUPBCERF, 2000; Cook, 2004; Joerger, 2003; Witte, 1998). Thus, growth promoting substances (growth promotants) were therefore produced to help remedy the situation.

2.2.1. Growth promotants/ Growth promoting substances

Growth promotants (Animalsmart, 2013) are substances which are used to increase the efficiency of animal production by increasing weight gain and other product output. Holtz (2009) grouped classical growth promotants into two main groups namely, hormonal growth promoters (HGP) and antibiotic or antimicrobial growth promoters (AGP).

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2.2.1.1. Hormonal growth promoters

Hormones are chemical messages that are naturally produced in the bodies of all animals and released into the blood by hormone-producing organs (endocrine glands) to effect changes or affect parts distant from where they are released (CUPBCERF, 2000 and Stephany, 2010). According to Stephany (2010) and VCHTSES (2006), hormones occur naturally in animals and are responsible for almost all the actions that are taken by all individuals. The use of hormones in boosting growth can be traced as far back as the late 1930s when research indicated that a substance drawn from the pituitary gland (later identified as bovine growth hormone) of cows when injected into other cows resulted in more milk yield (CUPBCERF, 2000). Estrogen was also found to be responsible for improving growth in poultry (CUPBCERF, 2000). Stephany (2010) further indicated that hormone use in animals gained more roots after the chemistry behind hormones was understood and commercial quantities were made synthetically. The principle behind growth promotion with hormones is to discourage protein depletion and encourage protein synthesis in animals so that more lean muscles are gained from relatively less feed (CBB/NCBA, 2007). Squires (2003) further explained that, HGP promote growth by

shifting metabolism to direct more nutrients into muscle with fewer nutrients deposited into fat and excreted substances.

2.2.1.1.1. Classification of hormonal growth promoters

There are several schools of thought on the groupings of hormonal growth promoters but only two are discussed here. Gracey *et al.* (1999) grouped HGP into four. They are:

1. Natural Sex Hormones: This group consists of female sex hormones (oestradiol, oestrogen and progesterone) and male sex hormones such as testosterone.
2. Synthetic hormone-like growth promoting substances: This group makes up the bulk of HGP in animal production and is made up of 3 subgroups which are:
   a. Synthetic steroid androgens like nandrolone, norethandrolone and phenylpropionate.
   b. Synthetic non-steriodal oestrogens including Stilbene oestrogen/Diethylstilboestrol (DES), zeranol and trenbolone and
   c. Synthetic steroidal progestens such as Melengestrol acetate (MGA).
3. Peptide hormones- They are proteinaceous in nature and also possess the functions of hormones. Growth hormone (GH), growth hormone releasing factor and thyrotrophin-releasing hormone (TRH) are some of the examples of peptide hormones used in promoting growth in farm animals.
4. β-Adrenoceptor agonists (beta-agonists) - These are normally used in treating respiratory diseases and tocolysis in farm animals but are also abused in the altering of growth in animals. Known β-agonists used for such purposes include clenbuterol, mebaterol, cimaterol, ractopamine and salbutamol.

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CUPBCERF (2000) rather grouped HGP based on their chemical composition. This system of categorization divided HGP into steroids and peptides. Steroids according to Moss (1989) are made up of fats whilst peptides are made of protein. Also, this system of grouping HGP, as indicated by CUPBCERF (2000), helps in determining how the hormone is administered. Steroids remain active after they are eaten and are not broken down by digestive enzyme while peptide hormones are broken down by digestive enzymes in the stomach resulting in lost of the hormonal effect. Peptide hormone can therefore be administered to animals by injections or by implanting them under the surfaces of the skin.

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2.2.1.1.2. The ban on the use of HGP

The use of hormones in farm animal production has been criticized due to the detrimental effects they cause to humans, animals and the environment in general. The harmful effects of HGP have been stated in several literature and they include:

i. Disruptions of the endocrine function in humans and animals leading to the rampant occurrence of auto-immune diseases (Stephany, 2010).
ii. Breast, cervical, testicular, ovary, colon and other forms of cancer. Gracey *et al.* (1999) and Holtz (2009), for example, explained that daughters born to mothers given DES treatment developed cervical cancers later in life.
iii. Stephany (2010) also reported of cases of serious reproductive defects in aquatic life that came in contact with some residues from farm animals given hormone treatment; this has also been observed in humans.
iv. Holtz (2009) also explained that hormone treatments and frequent contact with residues from some hormones can also lead to feminization.

v. Substances such as clenbuterol has been reported to be responsible for poisoning in individuals eating meat from animals impregnated with such substances in China and Spain (Stephany, 2010).

vi. CUPBCERF (2000) and Gracey *et al.* (1999) further stated that from studies in Italy in 1977, steroid hormone residues from poultry products and beef in school meals were suspected for the enlargement of breasts in very young girls and development of breasts in boys.

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Because of these developments, several countries notably Holland, Belgium and Denmark shunned the use of HGP in 1961, 1962 and 1963 respectively (Holtz, 2009). Also, all Benelux countries banned the use of all HGP in 1973. The European Commission (now European Union, EU) also abolished the use of all HGP in 1989. Currently only very few HGP are allowed in animal production for growth promotion and some other therapeutic use. CBB/NCBA (2007) and Stephany (2010) have stated that the few hormones accepted in animal production are oestradiol, testosterone, progesterone, trenbolone and zeranol which are administered as implants in heifers and melengestrol acetate and ractopamine which are administered as feed additives. Ractopamine according to Stephany and Ginkel (1996) is the only hormone allowed in pig production in the US. Hormone treatment in poultry is strictly abolished in Canada, the United States and in all EU countries.

2.2.1.2. Antibiotic/ Antimicrobial growth promoters (AGP)

Antibiotics are chemical substances, produced wholly or partly by a microorganism; usually a fungus or bacterium; which have the capacity to inhibit the growth of or to kill bacteria (Gracey *et al.*, 1999). Antibiotics, as the definition implies, are produced by the microbes themselves,

therefore they were long in existence before they became useful in human's fight against pathogenic bacteria.

2.2.1.2.1. Mechanisms of action of antibiotics

It is generally accepted that antibiotics have five basic ways or mechanisms by which they inhibit the growth of or kill both pathogenic and beneficial bacteria whenever they are administered (Rollins and Joseph, 2000). Each of these mechanisms is either structure or function specific or both and will therefore work or not work in a bacterium owing to the presence or absence of a structure or function.

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These 5 basic functions according to Bezoen *et al.* (1999), Rollins and Joseph (2000) and Soares *et al.* (2012) are as outlined below:

i. **The inhibition of cell wall synthesis-** This mechanism is normally displayed by antibiotics in the penicillin (Beta lactams) and vancomycin group. In this mechanism, susceptible bacteria are killed by the inhibition of synthesis of the peptidoglycan cell wall which provides the cell with rigid stability due to its highly cross-linked structure (Soares *et al.* 2012).
ii. **Translation or inhibition of protein synthesis-** Antibiotics in this group are bacteriostatic in nature and interrupt the formation of the initiation complex required for protein synthesis by binding to ribosomes within the bacteria. Antibiotics known to exhibit this kind of action include the aminoglycosides, tetracyclines and macrolides.
iii. **Curtailment of nucleic acid synthesis-** Kohanski *et al.* (2010) explained that antibiotics in the class of quinolones, metronidazole, bacitracin, etc. work by interfering with the maintenance of chromosomal topology by targeting DNA gyrase (Topoisomerase II) and

Topoisomerase IV. It has further been emphasized that their actions trap these enzymes at the DNA cleavage stage thereby preventing the strands from rejoining. Rifampicins on the other hand are known to inhibit RNA synthesis.

iv. **Antimetabolite activity/ the inhibition of metabolic pathways-** It has been speculated that some antibiotics work by preventing some metabolic reactions from taking place. Some of these actions include the prevention of the synthesis of folic acid required for the synthesis of purine and nucleic acid. Antibiotics known to exhibit this kind of action include the sulfonamides and trimethoprims. They are known to be bacteriostatic.

v. **The alteration of cell membrane-** This mechanism seeks to kill the bacteria by altering the permeability of the cell wall thereby granting the cytoplasmic contents of the bacteria chance to flow out. Antibiotics that exhibit this mechanism include those in the polymyxin and bacitracin class.

2.2.1.2.2. Antimicrobial or antibiotic resistance

The resistance to antimicrobials and other toxic chemicals, according to Bezoen *et al.* (1999) is an adaptation or survival mechanism exhibited by bacteria and other microbes in general to all forms of biochemical stress. Bacteria exhibit resistance to antibiotics and other toxins by mechanisms which according to Džidic *et al.* (2008) and Hooper *et al.* (2001) include:

i. Alteration or modification of target sites (penicillin binding proteins (PBP)) so that they are no longer bound by the antibiotics.

ii. Inactivation of the antibiotics by enzyme hydrolysis before they reach target sites.

iii. Modification of cell wall permeability such that they are either impermeable to the antibiotics or so large as to enhance the pumping-out of antibiotics which have already entered the cell.

iv. Absorption of insignificant quantities and

v. Target bypass

All of the above mentioned mechanisms of resistance are used in the two different forms of resistance which are:

1. Intrinsic/Inherent resistance or Insensitivity and
2. Acquired resistance.

a. Intrinsic resistance

Intrinsic resistance (MSU, 2011) is the innate ability of a bacterial species to resist the activity of a particular antimicrobial agent through its inherent structural or functional characteristics which allow tolerance of a particular drug or antimicrobial class. Russell and Chopra (1990) indicated that because this form of resistance is inherent or due mainly to some features of the bacterial, it cannot be passed on from one bacteria to another but only from a bacteria to its offspring. The mechanism of action of intrinsic resistance in bacteria, according to Bezoen *et al*. (1999); Ibezim (2005); MSU (2011) and Russell and Chopra (1990) are:

- The production of enzymes which inactivate the antibiotics e.g. *Klebsiella spp.* produces the enzymes Beta-lactamases that destroy ampicillin before the drug can reach the penicillin binding protein (PBP) target.

- The inaccessibility of the drugs into the cell components due to barriers impermeable to the antibacterial agent on the cell wall e.g. the outer membrane of Gram–negative bacteria can prevent the entrance of some β–lactams into the cell.
- The extrusion of the antimicrobial by chromosomally encoded active exporters.
- The lack of affinity of the antimicrobial for the bacterial target.
- Some bacterium like *E. faecium* are resistant to sulphonamide by utilizing other folic acid derivatives rather than depending on tetrahydrofolic acid which is inhibited by sulphonamide.

Intrinsic resistance is not much of a problem since it is the form of resistance that microorganism had even before the advent of antibiotics and other antimicrobial agents. This form of resistance or characteristics of individual species of bacteria serves as bases in the manufacturing of antibiotics.

b. Acquired resistance

This is the form of resistance which occurs when a microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible (MSU, 2011). Bezoen *et al.* (1999) stated that this form of resistance is raising concern worldwide since it does not only make years of research into drugs and monies spent, wasted but can also be the cause of several uncontrollable diseases and epidemics. Thus it calls for research into the development of new drugs which may also be ineffective on the pathogens with time and be even more expensive. There are two known mechanisms by which bacteria acquire this form of resistance. They are:

a. Mutation of chromosomes (Birošová and Mikulašová, 2005; Rutgers Biomedical and Health Sciences, 2013) and
b. The horizontal or lateral gene transfer.

i. Resistance through chromosomal mutation

Chromosomal mutation which is an inheritable alteration from the normal (Schleif, 1993) occurs in bacteria in a form of an alteration or change in the sequence of nucleotides in the DNA. Hooper *et al.* (2001) emphasized that, the potential for obtaining resistance by bacteria through mutating their chromosome is essential if the bacteria will survive the harsh environmental conditions in their delicate state. Therefore, bacteria and for that matter, microorganisms in general, keep on mutating with the objective of achieving a near perfect state which ensures survival from extinction. Chromosomal mutation, according to Bezoen *et al.* (1999), can occur at any time with or without the presence of an antibiotic; however, certain chemicals can facilitate the rate at which mutations occur (Birošová and Mikulašová, 2005). Dale and Park (2004) and Schleif (1993) indicated that mutation leads to the changes in the coding of portions of the DNA resulting in modification of the sequence of the amino acids of proteins which result in changes in the expressions of genes. As a result of changes in gene expression through mutation, previously bacteriostatic and bactericidal agents may no longer be as effective since their targets within the bacteria may no longer be in existence or there may even be over production of target sites such that the normal dosages may be ineffective (Birošová and Mikulašová, 2005).

Also, it has been indicated that mutation can change cell wall characteristics such that porins within the cell walls are no longer permeable to certain antibiotics (Birošová and Mikulašová, 2005). Mutation (Rutgers Biomedical and Health Sciences, 2013) may either deteriorate or

improve the condition of the bacteria. Some researchers according to (Rutgers Biomedical and Health Sciences, 2013) emphasized that; some form of mutation can even help to control some bacteria. It is worth emphasizing that this form of acquisition of resistance is not very important since it can occur with or without an antibiotic.

i. Resistance through horizontal or lateral gene transfer

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Horizontal gene transfer which is favoured by the presence of antibiotics occurs when bacteria pick up functional DNA from either the environment or from other bacteria (Bezoen *et al.*, 1999). This form of acquiring resistance is of more importance to scientists since according to Džidic *et al.* (2008) its occurrence is facilitated by the misuse of antibiotics by man and also by the nutritive/sub-therapeutic and therapeutic use of antibiotics in animal production. There are basically three forms of horizontal gene transfer: transformation, transduction and conjugation.

- Transformation

The uptake of naked DNA by naturally transformable bacteria is termed transformation (MSU, 2011). According to Bezoen *et al.* (1999), when a bacterium dies, its DNA if left behind intact in the surroundings can be picked-up by a competent bacterium which then incorporates parts of it into its own chromosome. In situations where the acquired DNA contains resistance genes, the bacterium then obtains the resistance and then passes it on to subsequent generations.

- Transduction

Bacteriophages are known to be viruses that infect and disintegrate bacteria. Studies however have shown that bacteriophages infect bacteria by only introducing its DNA into the bacteria (MSU, 2011) and may then break down the host's (bacteria) DNA into segments before

packaging some of the bacteria DNA and its own DNA. In some situations, according to Džidic *et al.* (2008), there may be errors in the packaging and the bacteriophage may then pick up only a bacteria DNA. After lyses of the bacteria, the bacteriophages may then go on to infect other bacteria but because some of the bacteriophages are made up of only bacteria DNA, they may enter into another bacteria and only form part of that bacterium's DNA. If the gene sequence transported by the bacteriophage contains resistance genes, then, according to Bezoen *et al.* (1999), the bacteria may obtain resistance. It is however worth stating that, this form of acquisition of resistance may be between only closely related bacteria since bacteriophages are known to have a narrow spectrum of host on which they depend (Bezoen *et al.*, 1999).

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- Conjugation

Bezoen *et al.* (1999) referred to conjugation as the transfer of DNA by direct cell-to-cell contact. Other researchers (Llosa *et al.*, 2002) prefer to define bacterial conjugation as the promiscuous DNA transfer mechanism between bacteria. Conjugation can occur between closely related and unrelated bacteria and therefore is said to be the main cause of the spread of antibiotic resistance among pathogenic bacteria (Dale and Park, 2004 and Llosa *et al.*, 2002).

Conjugation basically requires the direct contact between cells after which a channel (F-pilus) emerges between the 2 cells through which fragments of DNA are sent from a donor cell into a recipient cell (Babic *et al.*, 2008). Grohmann *et al.* (2003) stated that, when plasmids containing resistance genes are transferred and they are replicated and transcribed successfully, the recipient gains the resistance which can then be passed onto its offsprings. Table 1 summarizes how the different groups of antibiotics work, the specific strategies they use and how bacteria resist some of these strategies.

Table 1. Antibiotics, their mechanisms of action and mechanisms by which bacteria resist them.

Mechanism of action of antibiotics	Group/class of antibiotics	Example of antibiotics	Strategies of action	Resistant strategies by bacteria
Inhibition of cell wall synthesis	Beta-lactams	Penicillin	Distrupts the synthesis of peptidoglycan layer of bacterial cell wall	1. Enzyme action (Inactivation) 2. Target modification 3. Reduced intracellular accumulation
	Vancomycins	Vancocin		
	Bacitracin	Bacitracin, Bacitracin zinc, Methylene		
	Antimycobacterial agents	Streptomycin, Dapsone, Clofazimine		
Translation (inhibition of protein synthesis)	Aminoglycosides	Neomycin, streptomycin	Binding to bacterial 30S or 50S ribosomal subunit, Inhibiting the translocation of peptidyl-tRNA from A-site to P-site and also causing misreading of mRNA leaving the bacterium unable to synthesize protein vital to its growth.	1. Efflux pump 2. Target modification 3. Antibiotic modification through enzyme action. (Inactivation)
	Tetracyclines	Oxytetracycline, Chlorotetracycline		
	Chloramphenicol	Biomicin, Amphicol, Paraxin		
	Macrolides	Tilmicosin, Tylosin		
	Clindamycin	Dalacin, Lincocin, Daclin		
Inhibition of nucleic acid synthesis	Quinolones	Ciprofloxacin	Inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription.	1. Target modification 2. Reduced intracellular accumulation
	Metronidazole (Flagyl)	Metronidazole		
	Rifampicin	Rifadin, Rimactane		
	Bacitracin (Topical)	Bacitracin		
Antimetabolite activity	Sulfonamides and Dapsone	Sulfamethoxazole, sulfathiazole	Prevent the synthesis of folic acid required for synthesis of purine and nucleic acid.	1. Target modification
	Trimethoprim	Proloprim, Monotrim, Triprim		
	Trimethoprim-Sulfamethoxazole (synergism)	Bactrim, Co-trimoxazole, Septrin		
Alteration of cell membrane	Polmyxins (topical)	Neosporin	They displace bacterial counter ions which destabilize the outer membrane. (They normally alter the permeability of cell wall.)	1. Cell wall modification
	Bacitracin (topical)	Bacitracin		

Compiled from: Kohanski *et al.* (2010); MSU (2011); Rollins and Joseph (2000); Tenover (2006)

2.2.1.2.3. The ban on the use of antibiotic growth promoters in animal production

The intensive use of antibiotics in animal production had become a problem for man since it was observed that some antibiotic residues and some antibiotic resistant strains of bacteria were found in the food of man, and several researches according to Witte (1998), indicated that they were from animal protein sources like meat, eggs and milk, and even from the manure which are applied unto soils for crop production obtained from animals receiving antibiotic treatments and therapies. Also, Džidic *et al.* (2008) has intimated of the accumulation of evidence in support of the fact that antibiotic resistant bacteria from animals like pigs and poultry enter into the human food supply chain and end up colonizing the digestive tract and transpose resistance genes into the microflora within the intestines. Institutions were therefore setup to find ways of containing the problem of antibiotic resistance since it was making it difficult to treat some disease conditions in man, as well as making years of research in drugs and money invested into these researches, waste. In 1969 (Fuller, 1989), the Swann Committee came out with the recommendation which supported the abolishment of use of antibiotic growth promoters (AGP) used in the treatment of human diseases in animal feed. The UK was the first to put this strategy into practice in 1970 when it banned the use of penicillin and tetracycline as farm animal growth promoters (Buchanan *et al.*, 2008); Sweden rather followed UK's lead with a stricter measure by banning the use of all AGP in 1986 (Buchanan *et al.*, 2008; Dibner and Richards, 2005). Denmark and the Commission of the European Union as stated by Dibner and Richards (2005), banned the use of Avoparcin in 1995 and 1997 respectively; in 1998 Denmark banned the use of Virginiamycin whilst the EU gave the approval for the withdrawal of all antibiotics used in humans from animal growth promotion. Subsequently, Denmark banned all AGP in 1999 and the EU banned them in 2006. Dibner and Richards (2005) and Vondruskova *et al.* (2010) have

explained that with the current health awareness of consumers and pressure on food safety, there is the high likelihood of the US and other parts of the world banning the use of all AGP and therefore there is the need to investigate and find the best alternative to AGP which will also be safe within the food chain.

2.3. Some alternatives to antibiotics and hormones in growth promotion and health improvement

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Research into alternatives for antibiotic and hormonal growth promoters (AHGP) in animal production has become very active with various scientists trying to find the best possible alternative which will be cheaper and also very safe. Several products have been tested according to Vondruskova *et al.* (2010) but most of them have not yielded very concrete results. Some tested alternatives to antibiotic and hormonal growth promoters include:

a. Clay adsorbents
b. Organic acids/acidifiers
c. Botanicals or phytobiotics
d. Dietary or exogenous enzymes
e. Prebiotics
f. Synbiotics and
g. Probiotics

2.3.1. Clay adsorbents

Jacela *et al.* (2010a) outlined that the main rationale behind feeding animals with clay adsorbents is that consumption of feeds devoid of toxic substances improves performance and therefore increases efficiency. Clay adsorbents are therefore added to the diets of animals mainly to adsorb mycotoxins which are produced by microorganisms unlike antibiotics and other antimicrobial

agents which kill microbes directly. It has been stated by Wan *et al.* (2013) and Wicklein *et al.* (2008) that mycotoxins especially aflatoxins are immunosuppressive, carcinogenic, mutagenic and teratogenic and furthermore their use may result in poor average daily gain, feed intake, poor rates of feed conversion, liver damage and ultimately death in animals. Also, Jacela *et al.* (2010a) has stated that microbes which produce these toxic substances get in contact with plants which eventually become feed, on the field and therefore almost every plant material used as feed is contaminated and with storage conditions which are favourable, growth increases and more mycotoxins are produced. Clay adsorbents are therefore provided in the diets of animals to reduce the quantities of mycotoxins which would have otherwise been poisonous to animals. It has been stated (Galan 1996; Trckova *et al.* 2004; Wilson 2003) that supplementation of animal diets with clay adsorbents aside their detoxification ability may also perform the following functions in the animal:

1. Alleviate gastrointestinal upsets including diarrhoea and bloat.
2. Some clay pellets given to domestic animals may also supplement mineral nutrition.
3. Also, clay adsorbents have proved to reduce excess acidity in the digestive tract of farm animals and man.
4. It has been reported that clay adsorbents can also absorb some of the unpalatable compounds from animal feed and therefore improve feed intake.

Kannewischer (2006), citing several literature, indicated that there are several clay adsorbents which have yielded positive results under experimental conditions in different kinds of animals. Hydrated sodium calcium aluminosilicates (HSCAS) for example improved growth in aflatoxin-challenged chicken, turkeys, rats and pigs. Also, HSCAS supplementation in pregnant rats fed aflatoxin-contaminated diets resulted in maternal and foetal development similar to a control.

Calcium montmorillonite supplementation has also resulted in diminished effects of mycotoxins in chickens, rats and pigs. Furthermore, bentonite improved growth in rats and pigs. Nonetheless, it has been reported that excessive addition of clay adsorbents like kaolin resulted in decreases in growth in some farm animals (Trckova *et al.* 2004) which has been attributed to the ability of some clay compounds to bind or adsorb minerals limiting their bioavailability. The site from which clay used in making some of these clay adsorbents are obtained can also influence how wholesome they are. Jacela *et al.* (2010a) for instance indicated situations where clay mined near an industrial site contained polychlorinated dibenzodioxins (PCDD) commonly called dioxin; a potent poison to man and animals.

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2.3.2. Organic acids/ Acidifiers

Doyle (2001) and Papatsiros *et al*. (2012) defined organic acids as carboxylic acids including fatty acids and amino acids of the general structure R-COOH containing one to seven carbon atoms. Doyle (2001) and Mroz (2005) further explained that these acids are widely distributed in plants and animals and are produced during microbial fermentation of carbohydrates in the large intestines. Organic acids are known to have beneficial effects like improved health and resistance to diseases, faster growth, increased efficiency of diet utilization and better carcass quality (Bosi *et al*., 2005; Mroz, 2005) in animals when added to their diets. These effects are achieved through mechanisms which include:

i. Direct killing or growth inhibition of bacteria through the diffused activity of undissociated organic acids across the cell membranes and the destruction of cytoplasm (Mroz, 2005). This activity has been observed in *Clostridia* and *Salmonella* when diets containing formic acid were fed to weanling pigs (Bosi *et al*., 2005).

ii. The reduction in gastric pH which controls gastrointestinal microbial composition (Papatsiros *et al*., 2012; Partanen and Mroz, 1999).

iii. The gastric hydrolysis of organic acids releases H^+ ions which actuate pepsinogens and therefore improve protein digestion (Mroz, 2005).

iv. Organic acids also accelerate epithelial cell growth and mucosal development which ensures increased absorption (Mroz, 2005).

v. Acidifiers also act as precursors for the synthesis of non-essential amino acids, DNA and higher lipids required for growth (Mroz, 2005; Partanen and Mroz, 1999).

vi. Outside the animal's gut, organic acids have successfully been used to preserve feeds against fungal and bacterial infestation which is also another way of controlling disease causing organisms (Doyle, 2001).

Some organic acids may however reduce feed intake because of the pungent smells they produce (Mroz, 2005) which cause a diminution in feed palatability (Partanen and Mroz, 1999). Doyle (2001) also suggested that farmers do not patronize organic acids because of its corrosive effect on cement and galvanized steel feed troughs used in feeding animals.

2.3.3. Botanicals/ Phytobiotics

Most plants have resisted attacks from pest and pathogens for so many years and this resistance has been attributed to the aromatic antimicrobial substances which emanates from these plants (Jones, 2002). Kamel (2001) explained that this aromatic property of plants if tapped can be used as a perfect alternative for antibiotics in promoting growth in animals. Such medicinal plants and their extracts which are now added to animal feed to enhance performance are referred to as phytobiotics or botanicals (Vidanarachchi *et al*., 2005).

Plants like moringa (Ogbe and Affiku, 2012), neem (Zanu *et al.* 2011), ginger, garlic, etc. contain compounds which Soetan and Oyewole (2009) classified as secondary metabolites; like saponins, oxalates, trypsin inhibitors, etc. that are biologically active and can be used for medical purposes in both humans and animals. Unlike antibiotic growth promoters, which work by ensuring that there are no microorganisms but the animal alone making full use of feed given, phytobiotics use different mechanisms in meliorating growth in the animal. These mechanisms include:

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i. Flavour enhancement through their aroma which stimulates the animals' appetite (Jones, 2002; Lückstädt *et al.*, 2005). It is however worth mentioning that not all phytobiotic substances have this attribute since some phytobiotic plants like neem (Zanu *et al.*, 2011) are bitter and therefore does not stimulate feed intake.
ii. Phytobiotics are also known to stimulate digestive enzymes (in saliva and gastric juice) which facilitate proper digestion of feed (Jones, 2002).
iii. Direct anti-microbial effect by modulating the cellular membranes of microbes (Kamel, 2001; Vidanarachchi *et al*., 2005).
iv. Botanicals also stimulate the immune system by heightening the responses of the gut-associated lymphoid tissues (Vidanarachchi *et al*., 2005).
v. They contain potent anti-oxidants like the flavonoids and polyphenolic compounds which suppress the formation of reactive oxygen species and free radicals (Ogbe *et al.*, 2013; Patel, 2011).
vi. They exert prebiotic effects due to the fibre they contain (Jones, 2002; Kamel, 2001; Vidanarachchi *et al*., 2005).

vii. Phytobiotics also contains nutrients which also supplement nutrients provided in the animals'feed (Zanu *et al.,* 2011).

In Ghana, some phytobiotic plants like neem and Akakapenpen (*Rauvolfia vomitoria*) have been tested in broiler production and have yielded similar results as do the use of antibiotics and coccidiostats (Zanu *et al.,* 2011). The combination of several plants or herbs in growth promotion, according to Ogbe *et al.* (2013) is not advisable since it increases the concentration of anti-nutrients in the feed which may be detrimental to the animal's health. Jacela *et al.* (2010b) further explained that since phytobiotics have medicinal properties, an overdose of phytobiotics may be harmful to the animals as well as the consumers.

2.3.4. Dietary or Exogenous enzymes

Unlike other growth promoting substances aforementioned, exogenous enzymes promote growth in monogastric animals by supplementing the limiting levels of endogenous enzymes in breaking the complex bonds in plant materials, thereby making the nutrients in them readily available for digestion and absorption in the animal (Okai and Boateng, 2007). Dietary enzymes therefore helps improve digestibility of feed ingredients resulting in better feed conversion efficiencies (FCE) in farm animals. Alemawor *et al.* (2009) indicated that because Ghana is an agro-based country, there will be a lot of by-products left after every production season which can be used as feed for animals since the prices of conventional feed ingredients have reached unprecedented high levels.

Wenk (2000) also opined that the use of enzymes in animal feeds also helps to alleviate the risk of some digestive problems since certain anti-nutrients in feeds which would have bound with

some of the endogenous enzymes and resulted in digestive difficulties are rather broken down into forms which are no longer harmful but absorbable and useful in the animals' body.

The concept of adding exogenous enzymes to animal feed, according to Choct (2006) is not new but was not used since the limiting levels of technology made the extraction of enzymes expensive and therefore not advisable in animal nutrition. Also, Cunha *et al.* (2007) explained that enzymes were not used because there were no technologies to withstand the acidic conditions within the stomach and also the enzymes produced earlier were not thermostable.

In Ghana, there have been several studies on the use of enzymes (Alemawor *et al.*, 2009; Boateng *et al*., 2008; Boateng *et al*., 2013; Okai *et al*., 2000) which have yielded encouraging results. Also, surveys carried out by Boateng *et al.* (2011) indicated that quiet a substantial amount of poultry farmers have accepted and use exogenous enzymes in their feeding operations. It is withal worth mentioning that in using enzymes in feeding, the farmer should be sure of the particular complex plant materials to be broken down, since Okai (2010) explicated that enzymes are substrate specific and will therefore not work on the wrong substrate.

2.3.5. Prebiotics

The non-digestible food ingredients which are potentially beneficial to the health of the host due to their fermentable properties which may stimulate the growth and/or activity of one or a limited number of salutary bacteria in the colon or caecum according to Gibson and Roberfroid (1995) and Playne and Crittenden (1996) are termed prebiotics. Schrezenmeir and de Vrese (2001) elaborated further that the definition of prebiotics overlaps with that of dietary fibre with the only exception being its selective nature towards certain gut microbes. It has been stated that prebiotics can be extracted from natural sources like plants and milk; and also through partial

acid or enzymatic hydrolysis of polysaccharides or by transglycosylation reactions (Oku, 1996). Ammerman *et al.* (1988) and Patterson *et al.* (1997) stated that, prebiotics normally stimulate beneficial bacteria in the gut and hinder the growth of pathogenic microbes by complex mechanisms including:

i. The production of short chain fatty acids (SCFA), upon fermentation, which are useful to beneficial microbes but toxic to the pathogenic microbes.
ii. Prebiotics also increase the acidity of the hind gut thus selectively hindering the growth of pathogenic microbes.
iii. Also, prebiotics may influence balances in the ratio of beneficial and pathogenic bacteria by directly producing toxins which include bacteriocins which are bactericidal or bacteriostatic to pathogens.
iv. It has further been observed that, prebiotics hinder proliferation of pathogens by prevention of their adhesion to the intestinal mucosa simply through foxing their sugar receptors to attach to these oligosaccharides. These bacteria end up being excreted rather than attaching to the mucosal wall to proliferate (Lan *et al.*, 2005).
v. Also, prebiotics furnish beneficial microbes with nutrients and therefore ensure preferential growth, thus giving beneficial microbes the needed advantage in the competition for binding sites.

2.3.6. Synbiotics

The term "Synbiotics" originally was coined from the word synergy which implies that two products are working together for a greater effect. Therefore synbiotics refer to the combined use of prebiotics and probiotics (Lee *et al.*, 2009). Gibson and Robberfroid (1995) indicated that, not all products containing a combination of prebiotics and probiotics qualify to be called synbiotics

but rather when the prebiotic is a suitable substrate for the probiotic. This means that, the probiotics or DFM gets its nourishment from the prebiotic which then enhances its activity. Some functions of synbiotics as suggested by Collington *et al.* (1988) and Min *et al.* (1992) include increases in the composition of antibodies which boost the immune system, production of organic acids, decrease in the quantity of pathogenic bacteria, increase in enzymatic activity and decreased diarrhoea in weanlings especially when feeding synbiotics containing species of *Lactobacillus*. Also, Yang *et al.* (2005) observed improved probiotic and enzyme activity in the intestinal tract of rats when their feed was supplemented with synbiotics.

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2.3.7. Probiotics/ Direct Fed Microbial (DFM)

Preparations of live microorganisms which when administered in adequate quantities have beneficial effects on the health of the animal or person according to the World Gastroenterology Organization (WGO, 2008) are termed probiotics. Hamilton-Miller (2004), on the other hand, indicated that after the term probiotics was coined some 50 years ago, its definition has been either changed or upgraded on several occasions with the increase in the number of researches and findings on them. The author (Hamilton-Miller, 2004) therefore defined probiotics as live microorganisms administered in adequate amounts which confer a beneficial physiological effect and may also go a step further in preventing and curing disease conditions in the host. Gibson and Roberfroid (1995) also defined probiotics as microbial food or feed supplements which beneficially affect the host by improving its intestinal microbial balance. It is apparent that probiotics have been used extensively by humans for a long period of time but came into the limelight recently (Verna and Lucak, 2010). It has been stated that the effects of probiotics are beyond the inherent basic nutrition of the animal (Begley *et al.*, 2006) and researches are still being done to fully understand the actual ways and mechanisms by which these probiotics work.

Gathered evidence however, suggests that probiotics have been effective on improving growth rate and feed efficiency (Fuller, 1989; Fuller, 1995), the treatment of allergies (Hord, 2008), prevention of intestinal and urinary infections and neutralization of enterotoxins, relieving patients of constipation and lactose intolerance and also have anti-cholesterolemic effects (Neospark, 2013).

2.3.7.1. Attributes or properties of good probiotics

Not all feeds or foods containing microorganisms qualify to be called DFM or probiotics. It has been stated that some peculiar characteristics and qualities are required in accepting a product into the family of probiotics (Pal, 1999). Fuller (1989), Pal (1999) and Sekhon and Jairath (2010) outlined that a good probiotic/DFM should:

1. Not be pathogenic or toxic to the organism it is being administered to.
2. Be present as viable cells and in volumes not less than 30 CFU/g.
3. Possess the ability to survive and function well in the gut. All probiotic microbes are supposed to be bile and acid resistant.
4. Have the ability of being stable and capable of staying viable for long storage and harsh field conditions.
5. Affect host animals positively such as boosting immunity, meliorating growth and improving digestion.
6. Have good sensory properties.

Other attributes of DFM microbe are being gram positive, strain-specific and adhering to the lining of the intestines. It should however be emphasized that, some gram negative strains of probiotic bacteria have also been identified.

2.3.7.2. Mode of action of DFM

Fuller (1989), Playne (2003) and Rolfe (2000) outlined some mechanisms through which probiotics influence growth, improve health and protect their host. These mechanisms include:

1. Competitive exclusion: This is the use of space and nutrients by one organism, thereby denying the use of these commodities to another organism. Thus the beneficial microorganisms provided by the probiotic products use the food, space and even produce toxins which inhibit the growth of pathogens. It has been said that some of these salutary microorganisms produce VFA's which reduces the pH of the gut which results in unfavourable conditions for some of these pathogens.
2. Organic acid production: Strains of probiotics have been found to be associated with the production of a number of organic acids like acetic, lactic and formic acids, which inhibit intestinal pathogens. The organic acids produced also serve as energy sources to the animal or other beneficial bacteria. Also, it has been stated that some of these DFM produce micronutrients like vitamins which supplement what has already been provided in the feeds of animals.
3. Alteration of microbial metabolism: Some beneficial microbes provided in probiotics have been observed to produce enzyme(s) which aid in metabolism by either supplementing enzymes produced by the hosts' body or by producing enzyme which breakdown complex substances which would have otherwise gone undigested. Also, research findings suggest that some of these microbes produce enzymes which interfere with the metabolic activities of some pathogens whilst some absorb and reduce the enzymatic secretion produced by pathogens within the host. Rolfe (2000) for example indicated that some bacteria are associated with enzymatic repair of cells. Also,

Hamilton-Miller *et al.* (2003) reported that probiotics stimulate the activity of host enzymes.

4. Stimulation of immunity: The addition of probiotics to the diets of animals has been associated with the increased activity of the immune system. Fuller (1989) reported of increases in the levels of antibodies and increased activity of macrophages upon the administration of probiotics. Wu (2006) also suggested that probiotics influence the immune system by increasing the concentration of IgG, macrophagic and phagocytic activity of peripheral blood monocytes and granulocytes and white blood cells. Also, intestinal immune response has been associated with the use of probiotics. It has been emphasized that the use of probiotics containing organisms such as *L. acidophilus, L. casei, B. bifidum* and the *E. coli* strain, Nissle 1917 affects immune responses such as natural killer activity, cell mediated immunity, IgA production and apoptotic activity which reduce the occurrence of autoimmune diseases in humans and animals (Delcenserie *et al.*, 2008).
5. Production of antimicrobials: Bactericidal compounds that inhibit the growth of intestinal pathogens such as antimicrobials, bacteriocins and peroxides have been associated with DFM.
6. Reduction of toxic amines: Some strains of probiotics have been associated with the reduction in levels of amines which are produced by some microbes. Amines are toxic to animals and may compromise the wellbeing of animals.

2.3.7.3. Microorganisms used in DFM/ Probiotics

Several strains of microorganisms have been used in the manufacture of DFM and different microbes have been identified to affect hosts in diverse ways. DFM has been broadly classified

into two groups, bacteria and fungi. Most of these microbes used have been identified as non-pathogenic to animals and man (Fuller, 1989) and several species of microorganisms have been approved by the Food and Drug Administration (FDA, 1998) and the Association of American Feed Control Officials (AAFCO, 1998) for use in DFM products. Table 2 is a summary of the salutary microbes which have been approved for use.

Table 2. FDA and AAFCO approved microorganisms for use in DFM products.

Aspergillus niger	*Bifidobacterium infantis*	*Lactobacillus reuteri*
Aspergillus oryzae	*Bifidobacterium longum*	*Leuconostoc mesenteroides*
Bacillus coagulans	*Bifidobacterium thermophilum*	*Pediococcus acidilactici*
Bacillus lentus	*Lactobacillus acidophilus*	*Pediococcus cerevisiae(damnosus)*
Bacillus licheniformis	*Lactobacillus brevis*	*Pediococcus pentosaceus*
Bacillus pumilus	*Lactobacillus bulgaricus*	*Propionibacterium freudenreichii*
Bacillus subtilis	*Lactobacillus casei*	*Propionibacterium shermanii*
Bacteriodes amylophilus	*Lactobacillus cellobiosus*	*Saccharomyces sp.*
Bacteriodes capillosus	*Lactobacillus curvatus*	*Streptococcus cremoirs*
Bacteriodes ruminicola	*Lactobacillus delbrueckii*	*Streptococcus diacetilactis*
Bacteriodes suis	*Lactobacillus fermentum*	*Steptococcus faecium*
Bifidobacterium adolescentis	*Lactobacillus helveticus*	*Streptococcus intermedius*
Bifidobacterium animalis	*Lactobacillus lactis*	*Streptococcus lactis*
Bifidobacterium bifidum	*Lactobacillus plantarum*	*Streptococcus thermophiles*

Source: ADM Alliance Nutrition Inc. (2013).

2.3.7.4. Bacteria used in DFM

Bacteria strains such as those of the *Bacillus* genera are being used in the manufacture of probiotics but not as widely as those from the LAB group. A strain of the unpopular bacteria; *E. coli* (*E. coli* Nissile 1917) according to Fuller (1989) has also been identified and is among the pioneering microbes used in the making and study of probiotics. However, because of the presence of several pathogens within these groups and chances of plasmid transfers which can make them pathogenic, their use is being limited. This review will therefore concentrate on the common friendly bacteria employed in making DFM; lactic acid bacteria (LAB) and *Paenibacillus polymyxa* which will also be employed in the study.

2.3.7.4.1. Lactic Acid Bacteria (LAB)

Lactic acid bacteria produce several substances which help them to colonize the gut mucosa by preventing the attachment of pathogens. The substances produced include bacteriocins, antibiotics, lactic acid and peroxides (Lee *et al*., 2009). Noted lactic acid bacteria used in the making of probiotics include: *Lactobacillus sp.*, *Bifidobacterium sp.*, *Lactococcus sp*. *Streptococcus sp*. and *Enterococcus sp*. LAB are known to be among the very first groups of microbes that colonises the GIT of newly born piglets but with time they are substituted by pathogenic bacteria which include *E. coli* (Doyle, 2001).

a. *Lactobacillus species*

Lactobacilli are non-spore forming, non-flagellated, facultative anaerobic rod-shaped gram-positive bacteria. All bacteria belonging to this group are strictly fermentative. Doyle (2001) and Fuller (1989) opined that strains of *Lactobacillus* have been shown to improved growth of piglets and also decreased enteric disorders and diarrhoeal diseases and even appear to be more effective than some antibiotics. It has been indicated that about 56 species of *Lactobacillus* have been identified so far (Neospark, 2013).

b. *Bifidobacterium sp.*

Bifidobacteria are also non-spore forming, non-motile and gram positive anaerobic lactic acid bacteria which come in various shapes including short, curved, club shaped and bifurcated Y-shaped rods (Neospark, 2013). *Bifidobacteria* are known to be saccharolytic and produce lactic and acetic acid without the generation of carbon dioxide except during the breakdown of gluconate. They are also known to produce a number of antimicrobial elements which affects the

growth of pathogens. Because they produce organic acids, they reduce the pH of the gut making it difficult for pathogens to survive. Up to date, about 30 species of *Bifidobacterium* have been isolated.

c. *Enterococcus sp.*

Prominently known for its usefulness in the treatment of diarhoeal illnesses (Fuller, 1989), *Enterococci* are usually non-motile, non-spore forming, gram positive and facultative anaerobes. *Enterococci* form part of the normal microflora of the intestine of humans and animals. Doyle (2001) explained that they produce organic acids and toxins which inhibit the growth of pathogens.

2.3.7.4.2. *Paenibacillus polymyxa*

Paenibacillus polymyxa is a classical non-pathogenic plant growth promoting rhizobacterium known for its use in fixing nitrogen, sorption of phosphorus, enhancement of soil porosity and controlling of soil borne plant diseases (Anuraj *et al*., 2012). *P. polymyxa* according to MicrobeWiki (2012), is a gram positive, motile rod-shaped bacterium which is also a facultative anaerobe. It has further been stated by Lal and Tabacchioni (2009) that *P. polymyxa* produces metabolites like polymyxin E1 and Lantibiotics which have been proved to decrease pathogen colonization in farm animals notably poultry. Ravi *et al.* (2007) suggested that *P. polymyxa* has pathogenic activity against harmful man and animals pathogens such as *Vibro sp*. *P. polymyxa is also a* fermentative microbe that produces organic acids and H_2 as by-products which are also very useful in changing the pH of the gut thereby rendering the gut unsuitable for pathogen growth. It has also been pointed out that *P. polymyxa* has anti-fungal activity as well and is known to have deleterious effect against a broad spectrum of fungi. *P. polymyxa* also produces a lot of antimicrobial compounds which include polymyxin, fusaricidin and paenibacillin. Acetoin,

a compound known to be responsible for the unique flavor of butter and cigarette, and produced by *P. polymyxa* can stimulate feed intake in animals (MicrobeWiki, 2012).

2.3.7.5. Fungi used in DFM (*Saccharomyces sp.*)

Though non-pathogenic strains of fungi from the genera *Aspergillus* and *Trichosporon* have been used in the making of probiotics, species within the genus *Saccharomyces* have proved to be the most useful and most effective (MicrobeWiki, 2010). Santra *et al.* (1994) explained that feeding live cultures of yeast for example has been proved to improve the activities of rumen microorganisms including the enhancement of fibre degradation.

Saccharomyces which means "sugar fungus" are unicellular non-pathogenic fungi which are known for their use in the brewery and bakery industries. The use of yeast dates back in the early civilizations when it was used in making leavened bread. *Saccharomyces* cells according to MicrobeWiki (2010) are typically oblong spheroids with a prominent central vacuole and a small nucleus. Their cell walls are made of mainly glucans and mannoproteins. *Saccharomyces sp.* are known to be fermentative as well as respiratory.

Several species of *Saccharomyces* have been employed in DFM production but notable among them are *S. cerevisiae*, *S. boulardi* and *S. uvarum*. Chaudhary *et al.* (1995) and Kung (2001) emphasized that *Saccharomyces cerevisiae,* for example, have been observed to complement the enzymatic activity of hosts by providing a source of dietary enzymes. Also these fungi are known to supply B-vitamins which also help in the amelioration of growth. Munoz *et al*. (2005) indicated that *S. cerevisiae* and *S. boulardi* have been useful in the treatment of *Clostridium difficile*-related diarrhoea.

It is however worth mentioning that the use of *Saccharomyces sp.* in stressed animals and individuals with compromised immune systems should be with caution since some species have been found to be opportunistic pathogens. A typical example is the *Saccharomyces*-induced fungemia observed in female patients given probiotic therapy (Munoz *et al*., 2005).

2.3.7.6. Effects of DFM

There are several benefits that humans and animals can derive from the consumption of DFM or probiotics. It is worth stating that, most of the mechanisms behind these effects are not fully understood (Fuller, 1989) but studies are being done to find out the mechanism behind some of these effects. The effects of probiotics include their influences on growth promotion and immune system enhancement in humans and farm animals.

a. Effects of DFM on growth promotion in farm animals

It has been reported that the addition of DFM or probiotics to the diets of animals improves the rate of gain and the efficiency of utilization of feed (Abe *et al.*, 1995; Cho *et al.*, 1992 and Collinder *et al.*, 2000). These increases in weight gain and efficiency of gain, according to Fuller (1989) and Santos *et al.* (2005), are due to factors like the provision of a near pathogen-free gut environment which ensures that there is a reduction in, not only the amount of toxins produced in the gut, but also to ensure that pathogenic microorganisms do not get the chance to compete with farm animals for nourishment. Again, it has been stated that the addition of beneficial bacteria to animals feed have enzymatic effects on the feed which is given to animals. Playne (2003) for example indicated that probiotic bacteria secrete enzymes which ensure the breakdown of complex NSP which would have otherwise been voided out of the gut of monogastric farm animals because there are no suitable enzymes available for their digestion. Furthermore, the growth promoting effects of probiotics can be attributed to the fact that probiotic bacteria

synthesize organic acid and vitamins which are useful in the growth and health of animals. Several works done have reported of growth promotion effects of probiotics in farm animals. Rahimi (2009) for example, reported that the addition of probiotics to the diets of growing birds resulted in increased growth compared to those on a control diet with no probiotics. The author (Rahimi, 2009) reported average total weight gain of 2120g in probiotic-supplemented birds compared to 1903g in a control group and indicated that the differences between these means were statistically significant ($P < 0.05$). Also, Zulkifli *et al.* (2000) reported better gains in broilers fed diets containing DFM. In pigs, van Heugten *et al.* (2003) reported increases in daily weight gain when diets were supplemented with yeast culture. Furthermore, supplementation of broiler diets with 3 DFM products; Protexin, Primalac and Calciparine resulted in weight gains of 155.54g, 151.96g and 144.93g respectively in 6 week old broilers daily compared to 127.14g gained by birds on a control diet with no DFM (Shabani *et al.*, 2012). Chen *et al.* (2005) feeding (Duroc x Yorkshire) x Landrace crossbred pigs recorded 576g weight gain daily when they were fed a control diet but 623g in a probiotic supplemented group.

b. Effects of DFM on the immune system

Increased activity of the immune system of human and animals has been reported by several researchers (Isolauri *et al.*, 1993; Lee *et al.*, 2010 and Perdigon *et al.*, 1999) to be associated with probiotic supplementation. Though the mechanism behind the stimulatory effects of the immune system by DFM is not fully understood, it has been speculated that DFM improves the immune system by mediating the maturation and activation of dendritic cells (Hoarau *et al.*, 2006) which are known to initiate immune responses by presenting antigens to T-cells (Corthésy *et al.*, 2007). It has also been observed that DFM stimulates the immune system by increasing cytokine

expression, as well as, increasing phagocytotic activity and the composition of natural killer cells (Sanders, 2000).

Several cases of improved immunity have been associated with the presence of probiotic microbes in the guts of animals and humans. Isolauri *et al.* (2001) citing several sources indicated that children and mice supplemented with *Bifidobacteria* had higher levels of IgA and IgM in their blood compared to those on non-supplemented diets. Lee *et al.* (2010) reported of significantly higher levels of CD3, CD4 and CD8 in chickens supplemented with DFM. Wallace *et al.* (2012) recorded higher levels of WBC and lymphocytes in rabbits fed diets containing RE3™, a DFM product. In a sheep experiment, Kunavue and Lien (2012) indicated a significant ($P < 0.01$) increase in sheep red blood cell antibody when sheep on a control treatment and those on probiotic supplementation recorded values of 2.00 and 3.33 $\log^2$ respectively. Again, in broilers, serum antibodies against Newcastle disease virus increase significantly ($P < 0.05$) from 6.1 $\log 2^{-1}$ in a control group fed a basal diet with no added DFM to 7.2 $\log 2^{-1}$ in a DFM supplemented group (Rahimi, 2009).

2.3.7.7. RE3™ as a probiotic

RE3™ is a Direct-Fed Microbial product produced and distributed by Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada. Unlike some other DFM products, this product employs water as a carrier and thus comes in a liquid form. Basically, RE3™ is added to the feed of farm animals but can also be drenched to animals especially ruminants. Table 3 is a summary of the constituents of RE3™.

Table 3: Composition of RE3™

Constituents	Amount
Water	99.9%
Microorganisms	
Lactobacillus sp.	1 x 10^8 CFU/g
Bacillus sp.	4 x 10^{12} CFU/g
Saccharomyces cerevisiae	11 x 10^5 CFU
Minerals	
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 studies have been carried out on the effects of RE3™ on various growth, economic, and blood indices of pigs, poultry and rabbits; and on reproductive performance and immune status of rabbits in Ghana. Okai *et al.* (2010), working on the effects of RE3™ on pigs in Ghana, for example, reported of better average daily weight gain (ADWG) and FCR when weaner pigs were fed diets supplemented with RE3™. Furthermore, Wallace *et al.* (2012) reported better FCR and higher levels of WBC and lymphocytes in rabbits fed RE3™-supplemented diets compared to those on basal diets with no RE3™ supplementation. Again, Osei *et al.* (2013) reported higher birth weights in kits born to does on DFM supplementation. Earlier, Dei *et al.* (2010) had reported better FCR in starter broilers upon the inclusion of RE3™ in their diets. Again, Dei *et al.* (2010) indicated that the addition of RE3™ to the diets of broilers reduced the cost of medication by 32.4%. However, Amoah (2010) reported that the addition of RE3™ to the diets of pigs did not significantly ($P > 0.05$) improve growth and FCR of pigs. In broiler chickens, Bonsu *et al.* (2012), reported better FCR upon RE3™-supplementation compared to a control diet with no RE3™ (2.49 vs 2.74 respectively). Furthermore, the authors (Bonsu *et al.*, 2012) reported

higher weight gains in RE3™-supplemented broiler compared to those on a control (2002g vs 1762g). The supplementation of broiler diets with RE3™ again resulted in no mortalities compared to an average of 6 mortalities for broilers in a control group (Bonsu *et al.*, 2012).

2.4. Inferences from Literature Reviewed

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Though the domestication of animals opportune man easy access to animal products, the rise in population and the change in lifestyle meant there was a need to find ways of producing animals quicker to reduce cost of production so as to obtain substantial profits and also ensure consumers have access to cheap animal products. The AHGP were found to be one of the effective additives which could serve this purpose and at the same time protect animals from disease infections. It has however been observed that the addition of hormones and sub-therapeutic doses of antibiotics to animal feed increases chances of developing resistant bacteria strains which may be harmful to the well-being of man and animals alike. Also the risk of cancers and several other diseases have been noted. Legislations have therefore been enacted in some parts of the world especially Europe to curb the use of such AHGP. Because of the nature of the world market, there are speculations that these laws on non-antibiotic and hormone use in growth promotion may spread to the rest of the world and therefore warrant the search into products that can successfully replace antibiotics without any harmful effects on humans and the animals they are used on.

Several alternative products have been tested but in Ghana one of the most promising products are probiotics which have been evaluated based on their influence on growth performance, carcass characteristics, blood profile and economies of production in pigs and poultry. It is worth

emphasizing that no work has been done on the influence of probiotics/DFM on reproductive performance and its prolonged usage in pigs in Ghana. Again, Wallace *et al.* (2012) indicated that though the addition of RE3™ increased the levels of WBC and lymphocytes, it will be advisable to study the immunoglobulin content of blood to find out if truly RE3™ has effects on the immune responses of animals. Furthermore, new forms of probiotics are being developed daily. For example, Basic Environmental Systems and Technology (BEST), Inc., Alberta, Canada, the manufacturer of RE3™, is at the verge of introducing two more DFM products (RE-3 PLUS and P3) into the market and as such researches must be carried out to see their effects on farm animals.

Therefore, this study sought to identify the effects of three DFM products on the growth performance, haematology, blood serum biochemistry, immunology, gut microbiology and reproductive performance of gilts and the growth performance and blood composition of their progeny.

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CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Duration, site and phases of the experiment

The experiment which lasted a period of thirty-two (32) weeks (May, 2012 to January, 2013) was conducted at the Livestock Section of the Department of Animal Science, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The location had a maximum and minimum temperature range of 34.5 and 19.7ºC and a relative humidity range of between 34 and 87%.

The experiment comprised of two phases namely:

- Phase 1: Grower-finisher phase of gilts which spanned a period of 12 weeks.
- Phase 2: Gestation and Lactation phase.

3.2. Experimental Diets-General

Three diets were formulated and used at the different stages of the experiment. The diets and the stages at which they were used are as follows:

i. A 23 % CP creep / pre-starter diet (D1) which was given to piglets two weeks after they were farrowed.

ii. An 18 % CP diet which was fed to growing gilts and lactating sows (D2) and

iii. A finisher diet of 16 % CP which was fed to gestating gilts (D3)

Water was provided *ad-libitum* at both phases of the experiment.

Table 4 shows of the compositions of the diets used at the various phases of the experiment.

3.3. Treatments

There were four treatments in the experiment. These treatments are as listed below:

- Treatment 1 (T1) - no inclusion of DFM (Control)
- Treatment 2 (T2) - 1ml RE3™ per kg feed
- Treatment 3 (T3) - 1ml RE3 PLUS (a fermented product of RE3™) per kg feed
- Treatment 4 (T4) - 1ml RE3™ + 0.5ml P3 (a *Paenibacillus polymyxa* based DFM) per kg feed

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Table 4: Composition (%) of the various diets

	Diets*		
Ingredients	**D1**	**D2**	**D3**
Maize	54	58	62
Wheatbran	10	23.5	23
SBM	24	11.5	8.5
Fishmeal	10	5	4.5
Vit/min premix$^{\infty}$	0.25	0.25	0.25
Oyster shell	1	1	1
Common salt	0.25	0.25	0.25
Dicalcium phosphate	0.5	0.5	0.5
Total	**100**	**100**	**100**
Chemical composition (Calculated)			
Crude protein (%)	23.02	18.02	16.1
DE (Kcal/kg)	3305.60	3195.04	3191.53
Crude fibre (%)	4.12	5.30	5.20
Calcium (%)	0.99	0.83	0.79
Phosphorus (%)	0.78	0.80	0.74

**D1, D2 and D3 refer to a 23% CP creep feed, an 18% CP feed for growers and lactating animals and a 16% CP feed for finishers and gestating pigs respectively.*

$^{\infty}$ Vitamin premix per 100kg diet: Vitamin A(8x 10^5U.I), Vitamin D3 (1.5x10^4 U.I); Vitamin E (250mg); Vitamin K (100mg); Vitamin B2(2x10^2mg); Vitamin B12 (0.5mg); Folic acid (50mg); Nicotinic acid(8x10^2mg); Calcium panthotenate (200mg); Choline (5x10^3mg).Trace elements:Mg(5x10^3mg); Zn(4x10^3mg); Cu(4.5x10^2mg); Co (10mg); I (100mg); Se(10mg). Antioxidants: Butylated hydroxytoluene (1x10^3mg).Carrier: Calcium carbonate q.s.p (0.25kg).

The various DFM products were added to the feed of the pigs and mixed thoroughly daily before being fed to them.

3.4. PHASE 1- GROWER-FINISHER PHASE-GILTS

3.4.1. Animals, Design and Duration of the Experiment

Sixteen (16) Large White gilts with an average initial weight of 41.66 kg were randomly allocated to the four dietary treatments in a completely randomized design (CRD). Each treatment consisted of four gilts with a gilt representing a replicate. Thus, there were four replicates for each treatment in this experiment. This phase ended for every gilt after it attained a set live weight of 80±0.5kg.

3.4.2. Housing

The gilts were housed in a barn built with cement-blocks and with a concrete flooring. The barn had corrugated aluminium roofing sheets with two rows of pens separated by a 120 cm wide aisle, which provided access to all the pens. Each pen measured 365 x 300 cm and had a 120 cm high dwarf wall. The pens had in-built feed and water troughs (180 x 40 cm) moulded with concrete.

3.4.3. Feeding

Growing gilts were offered a diet containing 18% CP at a level of 4% of their body weight and the quantity of feed given to each animal was adjusted weekly after the gilts have been weighed till they attained the set live weight of 80±0.5kg.

3.4.4. Management

All pens were cleaned and disinfected before the start of the experiment. Subsequently, floors of the pens and water troughs were washed daily and fresh water provided every morning. Feeding troughs were also cleaned daily before feed was provided. Also, gilts were washed with Acaracide[1] at the beginning of the experiment and also at the sixth week of the experiment to destroy ectoparasites, if any. Before the start of the experiment, all gilts were tagged with plastic ear tags for easy identification.

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3.4.5. Blood and Faecal sampling

Blood samples were taken from the gilts at the start of the experiment and then on the last week of the phase (week 12) of the experiment through the jugular vena cava as described by Joslin (2009). Blood sample from each gilt was stored in 2 vacutainers: one containing Ethylenediaminetetraacetic acid (EDTA) and the other containing a gel activator. The blood samples stored in EDTA were used for haematological analysis whilst the serum collected from samples collected in the vacutainers containing clot activator was used in the biochemical and immunological analysis of the blood.

Faecal samples were also taken directly from the rectum and stored in 10ml containers for analysis on the gut microbial profile.

[1]Acaracide 10% - Cypermethrin-high CIS emulsifiable concentrate. Dosage: 1-2ml per litre of water. Supplied by: Afayad Animal Care- Kumasi, Tel. 0243737743.

3.5. PHASE 2-GESTATION AND LACTATION PHASE

3.5.1. Mating

After each gilts had attained a body weight of 80±0.5kg, it was monitored for signs of oestrus and were sent into a boar's pen for mating upon showing signs of standing heat. Mating was repeated 24 hrs. After the first mating, gilts were monitored and mated again in the event of a recurrence of heat.

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3.5.2. Feeding

A 16% CP diet was offered to the gilts at a level of 3% of body weight, and the feed allowance was restricted to 2kg/gilt/day from successful mating until farrowing. After farrowing, sows were allowed 5kg of an 18% CP diet. Also, a 23% CP pre-starter diet was provided to the piglets, two weeks after they were farrowed. Free creep feed was offered *ad libitum* daily.

3.5.3. Housing and Management

Gilts were moved into pens with similar dimensions as those used in the growing-finishing phase, but these had creep partitions and bedding in the form of wood shavings, two weeks prior to the expected farrowing date. Bedding was replaced when wet. All pregnant gilts were given the opportunity to exercise for about 10 minutes once every week. All piglets received an Iron dextran injection[2] within 3 days after they were farrowed. Also, all the piglets were identified with ear notches within 24 hrs after they had been farrowed. Pens were cleaned daily before feeding was done. Some health problems were also recorded at this phase of the study. These problems and how they were treated are as follows:

[2] Iron dextran injection- Ferro Dextrano 10% Sanphar, 1ml contains 100mg elementary iron (in dextran form). Dosage 2ml on 3rd day of life IM. Manufactured by Sanphar, Postal No. 8037-CEP 13058-971 Campinas/SP-Brazil. www.sanphar.com

1. Three gilts (one each from treatments T2, T3 and T4) had ruptured and inflamed vulvas during farrowing and were each administered with Dexamethasone injection[3].

2. One gilt from the Control (T1R4) showed signs of anorexia after farrowing and was administered with Multivite[4] for 3 days.

3.5.4. Weighing and Blood sampling of piglets

Body weight was taken for all piglets within 12 hours of birth and weekly thereafter. Blood samples were however collected from 2 randomly selected piglets in every set of litter within 24 hrs after birth and subsequently after every week until the piglets were weaned using the jugular venipuncture technique described by Joslin (2009). The blood samples collected within the first 2 weeks after farrowing were stored in vacutainers containing Ethylenediaminetetra-acetic acid (EDTA) whilst those samples collected on the third and fourth week were each divided into two and stored in 2 vacutainers; one containing EDTA and the other containing a gel or clot activator. The blood samples stored in EDTA were used for haematological analysis whilst serum collected from samples collected in vacutainers containing clot activator were used in the biochemical and immunological analysis of the blood.

[3] Dexamethasone injections- 2mg Dexamethason as sodiumphosphate/ml Dosage: 2-5mg per pig for 3 days IV or IM. Manufactured by Dopharma B.V., Zalmweg 24, 4941 VX Raamsdonksveer, The Netherlands. www.dopharma.com

[4] Multivite-1 ml contains: 50,000 i.u. Retinyl palmitate; 25,000 i.u. Cholecalciferol; 4mg Alpha-tocoferol acetate; 2.5mg Thiamine HCl; 2mg Riboflavin sodium phosphate; 1.25mg pyridoxine HCl; 0.03mg Cyanocobalamin; 2mg Ascorbic acid; 12.5mg Nicotinamide and 3mg D-Panthenol. Dosage: 4-5ml per sow IM or Subcutaneous. Manufactured by DutchFarm Veterinary Pharmaceuticals, DutchFarm International B.V., Nieuw Walden 112, P.O.Box 10, Nederhorst den Berg, Holland. www.dutchfarmint.com

3.6. Parameters measured

The parameters taken in this experiment were:

i. Feed intake

A Camry scale[5] was used to weigh feed daily for the gilts. Also, leftover feeds were weighed and the difference between the quantities of feed given and that left was considered to be the quantity of feed consumed by a pig. The quantities of feed consumed daily were used in computing for weekly and the total feed intake. Feed consumed by the piglets was however measured with a Master Chef digital kitchen scale[6]. The formula used was as follows:

Feed intake or Feed consumed (kg) = Feed offered (kg) – Feed Leftover (kg)

ii. Weight gain

Gilts were weighed weekly using a Gascoigne precision scale[7] during the grower finisher phase. Piglets were weighed on the next morning after they were farrowed and subsequently after every week until weaning with a camry scale. Weight gain was considered to be the differences between the initial and final weight after a phase as shown in the formula below:

Weight gain (kg) = Initial weight (kg) – Final weight (kg)

iii. Feed Conversion Ratio (FCR)

The efficiency of utilization of feed was calculated as the ratio of total feed consumed to the total weight gained by a pig. i.e.

[5] Camry scale (50g x25kg): Made in China.

[6] Master Chef digital kitchen scale (1gx5kg): Made in China.

[7] Gascoigne Precision Scale (200kgx500g): Manufactured by Precision Weighers, Reading, England.

$$FCR = \frac{\text{Feed consumed}}{\text{Weight gain}}$$

iv. Feed Cost and Economy of Gain

The cost of feed was computed by using prevailing market prices of the various commodities. Also, the cost of the DFM was added to the cost for dietary treatments T2, T3 and T4. Cost per kg weight gain was calculated by multiplying the cost of a kg of feed by the efficiency of utilization of the feed.

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Cost per gain (GH ¢) = FCR x Feed Cost (GH ¢)

3.7. Blood assays and gut microbial analysis

Haematological parameters were determined using an automatic haematology analyser. Total serum protein was determined by the Biuret method described by Kohn and Allen (1995) whilst albumin content was analysed using the Bromocresol Green method (Peter *et al.,* 1982). Globulin content was extrapolated for as the difference between the total protein and the albumin content. CD4 counts were determined by flow cytometry which is described as gold standard by Rungata (2008). CD3 counts were measured using a FACS count^{TM} (Becton Dickinson) system. IgA and IgM compositions were determined by the enzyme-linked-immunosorbent serologic assay (ELISA) procedure as described by Granstrom *et al.* (1994).

Faecal samples collected were serially diluted and known quantities were inoculated into Plate Count Agar in petri dishes. These samples were then incubated at a temperature of 35°C for 24 hours after which colonies formed were countered with the aid of a colony counter.

3.8. Statistical Analysis

All data collected were subjected to analysis of variance technique described in the Genstat 12th Edition (2009) and differences between treatment means determined by the Least Significant Differences (LSD).

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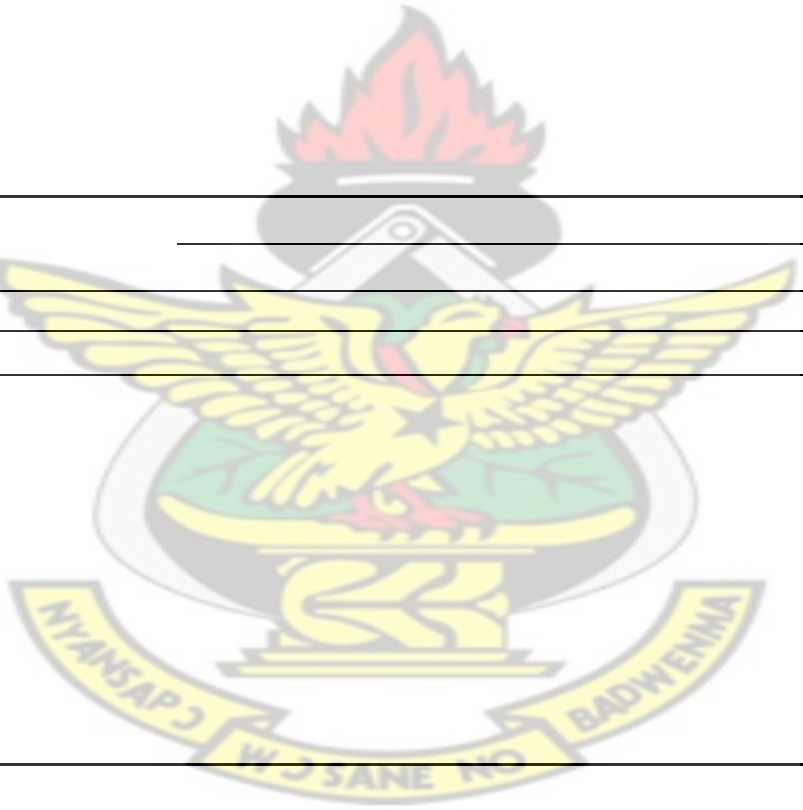

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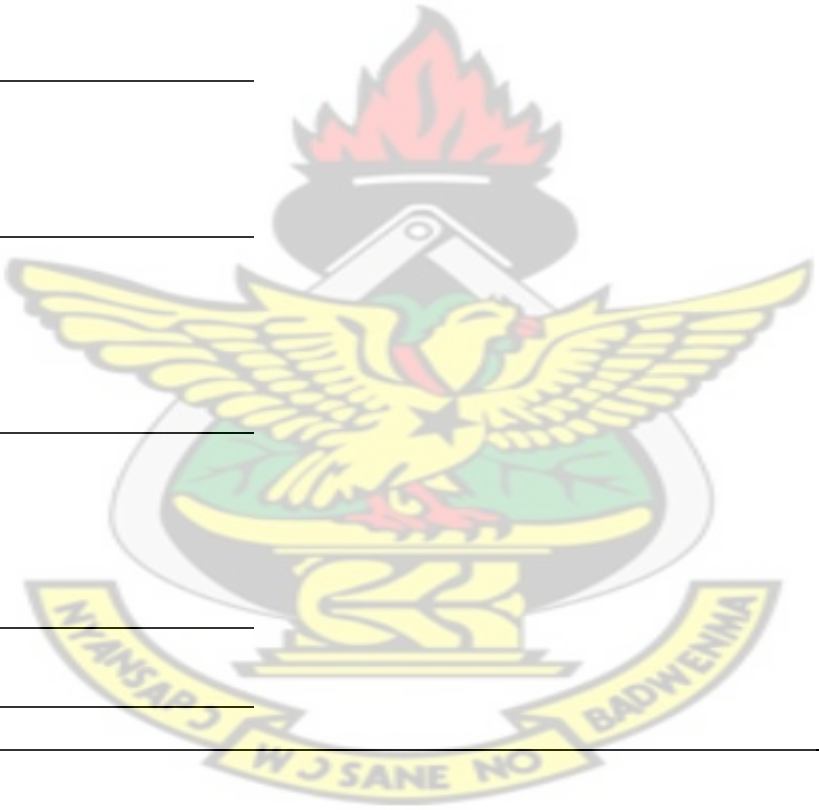

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CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

It can be concluded that with the exception of serum total protein, the addition of the 3 different DFM products to the diets of gilts did not seem to influence their growth performance, gut microbial composition, blood composition and reproductive performance. The addition of all 3 different DFM products to creep feed did not also seem to influence feed intake and weight gain in piglets but resulted in reducing the concentration of haemoglobin in red blood cells (MCHC) of piglets in the second and fourth week after birth. Furthermore, DFM supplementation decreased the levels of RBC 2 weeks after birth but resulted in increased levels of monocytes. Platelet levels increased in piglets on treatments T2 and T4 after 2 weeks of being fed the creep feed. Generally all piglets recorded low levels of MCHC and high levels of MCV. The addition of the 3 different DFM products to the diet of piglets resulted in significantly ($P \leq 0.05$) higher IgM values compared to those on the Control diet.

6.2. Recommendations

It is recommended that further trials are done on these different DFM products and if possible with higher numbers of gilts. Also on-farm trials should be carried out to determine the effects of the DFM products under on farm conditions.

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8.0 APPENDICES

APPENDIX 1: ANOVA (GROWTH PERFORMANCE AND ECONOMICS OF PRODUCTION OF GILTS)

Variate: Body_weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	10.688	3.562	1.89	0.201
Residual	12	16.938	1.882		
Total	15	32.438			

Variate: Duration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	134.75	44.92	1.06	0.411
Residual	12	379.75	42.19		
Total	15	1653.75			

Variate: Feed_intake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	978.5	326.2	1.44	0.293
Residual	12	2031.7	225.7		
Total	15	5802.5			

Variate: Weight_gain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	9.297	3.099	0.48	0.702
Residual	12	57.766	6.418		
Total	15	299.484			

Variate: FCR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.32125	0.10708	1.86	0.207
Residual	12	0.51917	0.05769		
Total	15	0.94886			

Variate: daily_weight_gain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.009186	0.003062	1.20	0.365
Residual	12	0.023038	0.002560		
Total	15	0.054367			

Variate: daily_feed_intake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.011170	0.003723	2.89	0.095
Residual	12	0.011587	0.001287		
Total	15	0.193971			

Variate: initial_weight					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.172	0.057	0.02	0.996
Residual	12	26.641	2.960		
Total	15	309.859			

Variate: cost_per_gain					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.24608	0.08203	2.01	0.166
Residual	12	0.48912	0.04076		
Total	15	0.73520			

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APPENDIX 2: ANOVA (BLOOD PROFILE OF GILTS (WEEK 1) PHASE 1)

Variate: Albumin_g_L

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		103.56	34.52	1.00	0.453
Residual	9	(3)	206.35	34.39		
Total	12	(3)	509.87			

Variate: Baso_%

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.7472	0.2491	1.14	0.407
Residual	9	(3)	1.3133	0.2189		
Total	12	(3)	2.0892			

Variate: CD3

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		616129.	205376.	3.63	0.122
Residual	7	(5)	226008.	56502.		
Total	10	(5)	489122.			

Variate: CD4

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		273456.	91152.	3.62	0.123
Residual	7	(5)	100707.	25177.		
Total	10	(5)	217677.			

Variate: EO_%

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		9.907	3.302	2.13	0.197
Residual	9	(3)	9.282	1.547		
Total	12	(3)	14.946			

Variate: GLOBULIN

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		324.9	108.3	0.63	0.622
Residual	9	(3)	1032.4	172.1		
Total	12	(3)	1456.3			

Variate: HB_g_Dl

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		17.021	5.674	1.01	0.451
Residual	9	(3)	33.735	5.623		
Total	12	(3)	112.277			

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Variate: HCT_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		127.21	42.40	0.84	0.521
Residual	9	(3)	304.04	50.67		
Total	12	(3)	1002.75			

Variate: IgA						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.03313	0.01104	0.75	0.568
Residual	8	(4)	0.07366	0.01473		
Total	11	(4)	0.14782			

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Variate: IgM						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.02049	0.00683	0.62	0.630
Residual	8	(4)	0.05484	0.01097		
Total	11	(4)	0.09422			

Variate: LYMPH_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		1340.8	446.9	4.22	0.063
Residual	9	(3)	635.6	105.9		
Total	12	(3)	1232.5			

Variate: MCHC_g_dL						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		39.894	13.298	1.40	0.330
Residual	9	(3)	56.869	9.478		
Total	12	(3)	103.108			

Variate: MCH_pg						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		25.082	8.361	1.40	0.331
Residual	9	(3)	35.834	5.972		
Total	12	(3)	68.617			

Variate: MCV_fL						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		262.52	87.51	4.77	0.050
Residual	9	(3)	110.14	18.36		
Total	12	(3)	269.13			

Variate: MONO_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		74.26	24.75	1.78	0.251
Residual	9	(3)	83.49	13.91		
Total	12	(3)	197.91			

Variate: NEUT_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		2291.7	763.9	3.93	0.072
Residual	9	(3)	1166.0	194.3		
Total	12	(3)	2476.7			

Variate: PLT_10_3_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		15546.	5182.	0.35	0.789
Residual	9	(3)	88157.	14693.		
Total	12	(3)	144463.			

Variate: RBC_10_6_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		4.563	1.521	1.16	0.401
Residual	9	(3)	7.898	1.316		
Total	12	(3)	23.644			

Variate: T_Protein_g_L						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		80.15	26.72	0.40	0.758
Residual	9	(3)	400.68	66.78		
Total	12	(3)	559.35			

Variate: WBC_10_3_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		152.58	50.86	2.26	0.182
Residual	9	(3)	135.28	22.55		
Total	12	(3)	585.71			

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APPENDIX 3: ANOVA (BLOOD PROFILE OF GILTS (WEEK 12) PHASE 1)

Variate: Albumin_g_L

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		255.59	85.20	1.71	0.242
Residual	11	(1)	398.65	49.83		
Total	14	(1)	787.25			

Variate: Baso_%

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		1.53870	0.51290	6.59	0.015
Residual	11	(1)	0.62222	0.07778		
Total	14	(1)	2.18000			

Variate: CD3

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		39913.	13304.	1.04	0.426
Residual	8	(1)	102373.	12797.		
Total	14	(1)	195719.			

Variate: CD4

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		17958.	5986.	1.05	0.421
Residual	11	(1)	45543.	5693.		
Total	14	(1)	85739.			

Variate: EO_%

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		1.1109	0.3703	0.56	0.654
Residual	8	(1)	5.2506	0.6563		
Total	14	(1)	7.5733			

Variate: GLOBULIN

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		51.10	17.03	0.36	0.783
Residual	11	(1)	377.94	47.24		
Total	14	(1)	535.93			

Variate: HB_g_Dl

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		15.464	5.155	1.97	0.198
Residual	11	(1)	20.982	2.623		
Total	14	(1)	36.580			

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Variate: HCT_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		135.53	45.18	2.18	0.168
Residual	11	(1)	165.67	20.71		
Total	14	(1)	319.60			

Variate: IgA						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.032683	0.010894	1.41	0.310
Residual	11	(1)	0.062000	0.007750		
Total	14	(1)	0.098893			

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Variate: IgM						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.012850	0.004283	0.56	0.655
Residual	11	(1)	0.060900	0.007612		
Total	14	(1)	0.085733			

Variate: LYMPH_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		2.740	0.913	0.65	0.604
Residual	11	(1)	11.204	1.400		
Total	14	(1)	21.724			

Variate: MCHC_g_dL						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		8.634	2.878	2.46	0.137
Residual	11	(1)	9.344	1.168		
Total	14	(1)	18.940			

Variate: MCH_pg						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		1.3120	0.4373	0.46	0.718
Residual	11	(1)	7.6122	0.9515		
Total	14	(1)	10.3240			

Variate: MCV_fL						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		50.60	16.87	0.65	0.604
Residual	11	(1)	207.15	25.89		
Total	14	(1)	289.27			

Variate: MONO_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.9317	0.3106	0.59	0.640
Residual	11	(1)	4.2300	0.5287		
Total	14	(1)	6.6293			

Variate: NEUT_%						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		20.380	6.793	2.65	0.120
Residual	11	(1)	20.486	2.561		
Total	14	(1)	59.197			

Variate: PLT_10_3_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		12838.	4279.	1.17	0.381
Residual	11	(1)	29342.	3668.		
Total	14	(1)	45343.			

Variate: RBC_10_6_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		4.895	1.632	1.41	0.310
Residual	11	(1)	9.274	1.159		
Total	14	(1)	15.186			

Variate: T_Protein_g_L						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		483.21	161.07	4.17	0.047
Residual	11	(1)	308.85	38.61		
Total	14	(1)	800.76			

Variate: WBC_10_3_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		32.088	10.696	1.70	0.243
Residual	11	(1)	50.222	6.278		
Total	14	(1)	112.273			

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APPENDIX 4: ANOVA (BACTERIAL PROFILE OF GUT CONTENT)

Variate: e_coli						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		4820.4	1606.8	2.54	0.110
Residual	11	(1)	6960.0	632.7		
Total	14	(1)	11278.1			

Variate: proteus					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	2067.2	689.1	1.00	0.426
Residual	12	8268.8	689.1		
Total	15	10335.9			

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APPENDIX 5: ANOVA (REPRODUCTIVE PERFORMANCE OF GILTS AND PIGLET GROWTH PERFORMANCE).

Variate: creep_feed_total intake						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		110072.	36691.	2.04	0.210
Residual	6	(6)	108066.	18011.		
Total	9	(6)	175608.			

Variate: daily_creep feed_total intake						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		561.59	187.20	2.04	0.210
Residual	6	(6)	551.36	91.89		
Total	9	(6)	895.96			

Variate: sow daily feed intake phase 2						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		9.610	3.203	2.22	0.186
Residual	6	(6)	8.650	1.442		
Total	9	(6)	13.792			

Variate: sow total feed intake phase 2						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		7534.	2511.	2.22	0.186
Residual	6	(6)	6781.	1130.		
Total	9	(6)	10813.			

Variate: sow weekly feed intake phase 2						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		470.87	156.96	2.22	0.186
Residual	6	(6)	423.83	70.64		
Total	9	(6)	675.82			

Variate: weekly creep feed intake						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		27518.	9173.	2.04	0.210
Residual	6	(6)	27016.	4503.		
Total	9	(6)	43902.			

Variate: piglet_wt_gain						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		10.869	3.623	3.51	0.089
Residual	6	(6)	6.194	1.032		
Total	9	(6)	11.858			

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Variate: ave_piglet wt_day_1						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.03256	0.01085	0.66	0.598
Residual	9	(3)	0.14851	0.01650		
Total	12	(3)	0.17844			

Variate: ave_ piglet wt_wk_1						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.1696	0.0565	0.30	0.827
Residual	6	(6)	1.1443	0.1907		
Total	9	(6)	1.2550			

Variate: ave_ piglet wt_wk_2						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		0.4892	0.1631	0.73	0.571
Residual	6	(6)	1.3432	0.2239		
Total	9	(6)	1.6922			

Variate: ave_ piglet wt_wk_3						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		3.4581	1.1527	1.86	0.237
Residual	6	(6)	3.7193	0.6199		
Total	9	(6)	5.7623			

Variate: ave_ piglet wt_wk_4						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		11.975	3.992	3.54	0.088
Residual	6	(6)	6.760	1.127		
Total	9	(6)	13.003			

Variate: percentage_mortality					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	4848.	1616.	1.16	0.376
Residual	9	12510.	1390.		
Total	12	17358.			

Variate: mortality						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		59.63	19.88	1.51	0.276
Residual	9	(3)	118.08	13.12		
Total	12	(3)	171.23			

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Variate: number_born						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		3.417	1.139	0.54	0.667
Residual	9	(3)	19.000	2.111		
Total	12	(3)	21.692			

Variate: number_weaned						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	3		60.74	20.25	1.77	0.222
Residual	9	(3)	102.75	11.42		
Total	12	(3)	154.77			

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APPENDIX 6: ANOVA (BLOOD PROFILE OF PIGLETS WITHIN 24 HOURS AFTER BIRTH)

Variate: Baso_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.019500	0.006500	1.17	0.369
Residual	10	0.055500	0.005550		
Total	13	0.075000			

Variate: EO_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.8793	0.2931	0.73	0.557
Residual	10	4.0100	0.4010		
Total	13	4.8893			

Variate: HB_g_dL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	15.952	5.317	0.81	0.517
Residual	10	65.731	6.573		
Total	13	81.684			

Variate: HCT_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	319.95	106.65	2.17	0.155
Residual	10	491.76	49.18		
Total	13	811.71			

Variate: LYMPH_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	33.37	11.12	0.38	0.772
Residual	10	295.81	29.58		
Total	13	329.18			

Variate: MCHC_g_dL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	6.596	2.199	0.59	0.635
Residual	10	37.247	3.725		
Total	13	43.844			

Variate: MCH_pg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	38.108	12.703	2.09	0.166
Residual	10	60.909	6.091		
Total	13	99.017			

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Variate: MCV_Fl					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	253.93	84.64	1.32	0.321
Residual	10	639.25	63.93		
Total	13	893.18			

Variate: MONO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.1605	0.3868	0.75	0.548
Residual	10	5.1767	0.5177		
Total	13	6.3371			

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Variate: NEUT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	37.04	12.35	0.36	0.785
Residual	10	345.43	34.54		
Total	13	382.47			

Variate: PLT_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	118488.	39496.	1.35	0.313
Residual	10	292399.	29240.		
Total	13	410887.			

Variate: RBC_10_6_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.6472	0.5491	1.09	0.396
Residual	10	5.0199	0.5020		
Total	13	6.6671			

Variate: WBC_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	25.21	8.40	0.43	0.734
Residual	10	194.08	19.41		
Total	13	219.29			

APPENDIX 7: ANOVA (BLOOD PROFILE OF PIGLETS 1 WEEK AFTER BIRTH)

Variate: Baso_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.001974	0.000658	0.08	0.967
Residual	9	0.070333	0.007815		
Total	12	0.072308			

Variate: EO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.1940	0.0647	0.12	0.945
Residual	9	4.7783	0.5309		
Total	12	4.9723			

Variate: HB_g_dL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.6234	0.5411	0.56	0.656
Residual	9	8.7197	0.9689		
Total	12	10.3431			

Variate: HCT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	13.202	4.401	0.45	0.722
Residual	9	87.695	9.744		
Total	12	100.897			

Variate: LYMPH_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	40.30	13.43	0.70	0.574
Residual	9	171.87	19.10		
Total	12	212.16			

Variate: MCHC_g_dL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	2.2256	0.7419	2.28	0.148
Residual	9	2.9237	0.3249		
Total	12	5.1492			

Variate: MCH_pg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	6.209	2.070	0.69	0.579
Residual	9	26.881	2.987		
Total	12	33.089			

Variate: MCV_fL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	87.06	29.02	0.51	0.684
Residual	9	510.10	56.68		
Total	12	597.16			

Variate: MONO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.3079	0.4360	1.08	0.407
Residual	9	3.6413	0.4046		
Total	12	4.9492			

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Variate: NEUT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	44.08	14.69	0.81	0.522
Residual	9	164.08	18.23		
Total	12	208.16			

Variate: PLT_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	80636.	26879.	0.56	0.655
Residual	9	431874.	47986.		
Total	12	512510.			

Variate: RBC_10_6_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.5583	0.5194	0.76	0.542
Residual	9	6.1132	0.6792		
Total	12	7.6715			

Variate: WBC_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	17.51	5.84	0.27	0.847
Residual	9	196.34	21.82		
Total	12	213.85			

APPENDIX 8: ANOVA (BLOOD PROFILE OF PIGLETS 2 WEEKS AFTER BIRTH)

Variate: Baso_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.031333	0.010444	1.98	0.175
Residual	11	0.058000	0.005273		
Total	14	0.089333			

Variate: EO_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	2.3193	0.7731	2.68	0.099
Residual	11	3.1767	0.2888		
Total	14	5.4960			

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Variate: HB_g_dL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	10.532	3.511	2.51	0.113
Residual	11	15.395	1.400		
Total	14	25.927			

Variate: HCT_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	167.02	55.67	2.96	0.079
Residual	11	206.77	18.80		
Total	14	373.80			

Variate: LYMPH_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	116.50	38.83	2.92	0.082
Residual	11	146.39	13.31		
Total	14	262.89			

Variate: MCHC_g_dL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	7.2952	2.4317	5.39	0.016
Residual	11	4.9608	0.4510		
Total	14	12.2560			

Variate: MCH_pg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	5.261	1.754	0.89	0.478
Residual	11	21.735	1.976		
Total	14	26.996			

Variate: MCV_fL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	150.91	50.30	2.37	0.126
Residual	11	233.52	21.23		
Total	14	384.43			

Variate: MONO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	3.1887	1.0629	9.07	0.003
Residual	11	1.2887	0.1172		
Total	14	4.4773			

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Variate: NEUT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	119.78	39.93	2.90	0.083
Residual	11	151.48	13.77		
Total	14	271.27			

Variate: PLT_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	25247.	8416.	0.69	0.575
Residual	11	133668.	12152.		
Total	14	158916.			

Variate: RBC_10_6_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	5.8650	1.9550	4.83	0.022
Residual	11	4.4548	0.4050		
Total	14	10.3198			

Variate: WBC_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	38.74	12.91	0.36	0.780
Residual	11	389.87	35.44		
Total	14	428.61			

APPENDIX 9: ANOVA (BLOOD PROFILE OF PIGLETS 3 WEEKS AFTER BIRTH)

Variate: Albumin_g_L					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	3.490	1.163	0.17	0.916
Residual	12	83.395	6.950		
Total	15	86.884			

Variate: Baso_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.004875	0.001625	0.33	0.805
Residual	12	0.059500	0.004958		
Total	15	0.064375			

Variate: CD3					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	288491.	96164.	0.24	0.865
Residual	12	4752275.	396023.		
Total	15	5040766.			

Variate: CD4					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	163716.	54572.	0.22	0.882
Residual	12	2994637.	249553.		
Total	15	3158354.			

Variate: EO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.5774	0.1925	0.79	0.524
Residual	12	2.9320	0.2443		
Total	15	3.5094			

Variate: GLOBULIN					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	56.98	18.99	0.78	0.528
Residual	12	292.30	24.36		
Total	15	349.28			

Variate: HB_g_dL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	11.608	3.869	0.47	0.708
Residual	12	98.590	8.216		
Total	15	110.198			

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Variate: HCT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	137.01	45.67	0.66	0.592
Residual	12	829.85	69.15		
Total	15	966.86			

Variate: IgA					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.01866	0.00622	0.21	0.888
Residual	12	0.35554	0.02963		
Total	15	0.37419			

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Variate: IgM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.05107	0.01702	0.45	0.722
Residual	12	0.45422	0.03785		
Total	15	0.50529			

Variate: LYMPH_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	22.90	7.63	0.57	0.648
Residual	12	162.10	13.51		
Total	15	185.00			

Variate: MCHC_g_dL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	2.965	0.988	0.16	0.918
Residual	12	72.165	6.014		
Total	15	75.130			

Variate: MCH_pg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	78.24	26.08	1.20	0.352
Residual	12	261.26	21.77		
Total	15	339.50			

Variate: MCV_fL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1124.4	374.8	2.01	0.166
Residual	12	2234.3	186.2		
Total	15	3358.8			

Variate: MONO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.1913	0.3971	0.92	0.459
Residual	12	5.1662	0.4305		
Total	15	6.3575			

Variate: NEUT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	25.93	8.64	0.50	0.692
Residual	12	209.45	17.45		
Total	15	235.38			

Variate: PLT_10_3_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Trt	3		92192.	30731.	0.53	0.673
Residual	11	(1)	642364.	58397.		
Total	14	(1)	725144.			

Variate: RBC_10_6_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	4.332	1.444	1.13	0.377
Residual	12	15.386	1.282		
Total	15	19.719			

Variate: T_Protein_g_L					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	25.69	8.56	0.71	0.564
Residual	12	144.54	12.05		
Total	15	170.24			

Variate: WBC_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	42.73	14.24	0.78	0.527
Residual	12	218.94	18.24		
Total	15	261.66			

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APPENDIX 10: ANOVA (BLOOD PROFILE OF PIGLETS 4 WEEKS AFTER BIRTH)

Variate: Albumin_g_L					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	66.12	22.04	1.72	0.213
Residual	13	166.98	12.84		
Total	16	233.10			

Variate: Baso_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.013294	0.004431	0.70	0.567
Residual	13	0.082000	0.006308		
Total	16	0.095294			

Variate: CD3					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	538498.	179499.	0.64	0.602
Residual	13	3636791.	279753.		
Total	16	4175289.			

Variate: CD4					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	475453.	158484.	0.86	0.488
Residual	13	2407341.	185180.		
Total	16	2882793.			

Variate: EO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	1.8337	0.6112	0.96	0.441
Residual	13	8.2875	0.6375		
Total	16	10.1212			

Variate: GLOBULIN					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	5.09	1.70	0.07	0.973
Residual	13	296.87	22.84		
Total	16	301.96			

Variate: HB_g_Dl					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	15.256	5.085	1.37	0.296
Residual	13	48.273	3.713		
Total	16	63.529			

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Variate: HCT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	221.86	73.95	1.28	0.324
Residual	13	753.50	57.96		
Total	16	975.36			

Variate: IgA					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.16078	0.05359	2.04	0.158
Residual	13	0.34177	0.02629		
Total	16	0.50255			

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Variate: IgM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.14033	0.04678	3.41	0.050
Residual	13	0.17847	0.01373		
Total	16	0.31880			

Variate: LYMPH_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	17.66	5.89	0.39	0.759
Residual	13	194.21	14.94		
Total	16	211.87			

Variate: MCHC_g_dL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	21.656	7.219	4.59	0.021
Residual	13	20.463	1.574		
Total	16	42.119			

Variate: MCH_pg					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	313.39	104.46	2.35	0.120
Residual	13	578.92	44.53		
Total	16	892.31			

Variate: MCV_fL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	2989.2	996.4	1.97	0.168
Residual	13	6560.2	504.6		
Total	16	9549.4			

Variate: MONO_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	0.2931	0.0977	0.18	0.905
Residual	13	6.8775	0.5290		
Total	16	7.1706			

Variate: NEUT_%					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	9.16	3.05	0.16	0.919
Residual	13	242.10	18.62		
Total	16	251.26			

Variate: PLT_10_3_ul						
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Trt	3		284728.	94909.	4.19	0.033
Residual	11	(2)	248972.	22634.		
Total	14	(2)	490652.			

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Variate: RBC_10_6_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	2.036	0.679	0.46	0.716
Residual	13	19.220	1.478		
Total	16	21.256			

Variate: T_Protein_g_L					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	45.86	15.29	0.53	0.668
Residual	13	372.84	28.68		
Total	16	418.70			

Variate: WBC_10_3_ul					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	3	141.10	47.03	1.06	0.400
Residual	13	577.55	44.43		
Total	16	718.65			