THE EFFECTS OF RE-3, A DIRECT-FED MICROBIAL (DFM) PRODUCT ON THE GROWTH PERFORMANCE, BLOOD PROFILE AND CARCASS CHARACTERISTICS OF PIGS.

BY

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

OF

MASTER OF SCIENCE

(ANIMAL NUTRITION)

FACULTY OF AGRICULTURE

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

OCTOBER, 2010.

DECLARATION

I, Kwame Owusu Amoah, 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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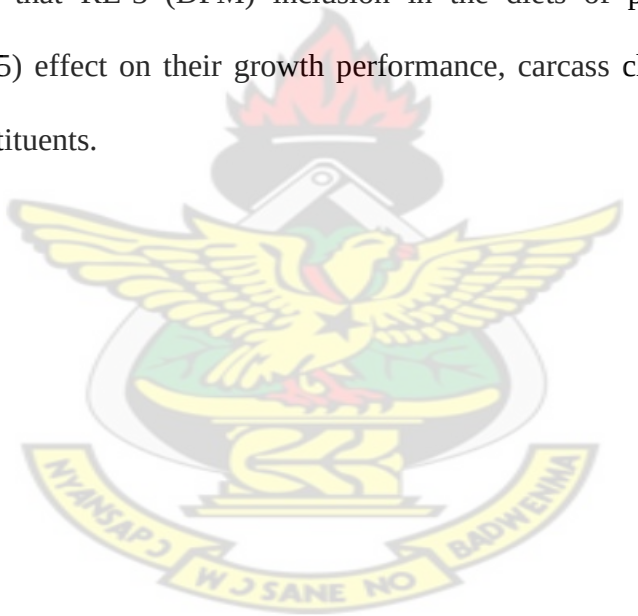
ABSTRACT

A feeding trial involving forty-eight (48) Large White starter pigs (24 barrows and 24 gilts) 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 21-week study was carried out to investigate the effects of Direct-fed microbials (DFM) on the growth performance, blood profile and carcass characteristics of pigs. Pigs with an overall mean initial liveweight of 10.38 kg, were selected and randomly allocated to three dietary treatments (designated as CONTROL, DFM-1, and DFM-2) based on sex and liveweight. Each treatment had sixteen (16) pigs and was replicated four (4) times and each replicate consisted of two (2) barrows and two (2) gilts. The experimental design used was Randomized Complete Block Design. The study was conducted in two phases, i.e. the Starter and Grower phases. The Starter phase lasted five (5) weeks and the diets offered each contained 18% CP whereas during the Grower phase, the diets offered contained 16% CP and its duration was from the sixth week until the end of the experiment. There was no DFM in the CONTROL diet. The DFM-1 and DFM-2 dietary treatments were the same as the Control diet but at feeding time 1.5 (DFM-1) and 3.0 ml of DFM (DFM-2)/kg diet were added. This rate of DFM inclusion was the same for the two phases of the study. Both feed and water were provided *ad-libitum.*

Pigs were weighed at weekly intervals and those attaining the specified weight of 70± 0.5kg on the weighing day were removed and slaughtered immediately for carcass characteristics and blood studies. Feed samples were collected for the two different phases for proximate analyses.

The DFM supplementation in the diets did not significantly ($P>0.05$) influence mean daily feed intake, daily weight gains, feed efficiency, and the number of days to slaughter. The full and empty GIT weights, dressed weight, dressing percentage, backfat thickness, loin eye area and the weights of the internal organs were also similar ($P>0.05$) The feed cost per kg weight gain was however significantly ($P<0.05$) increased at the higher inclusion level of DFM (i.e. 3.0 ml DFM/kg diet). The haematological and serum biochemical parameters were not significantly different ($P>0.05$) among the dietary treatments except for serum albumin.

It was concluded that RE-3 (DFM) inclusion in the diets of pigs did not have any significant ($P>0.05$) effect on their growth performance, carcass characteristics and most blood profile constituents.



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LIST OF ABBREVIATIONS USED

ABBREVIATION	DESCRIPTION
AAFCO	Association of American Feed Control Officials
AOS	Agaro-oligosaccharides
CDC	Center for Disease Control
CP	Crude protein
DFM	Direct-fed microbials
DM	Dry matter
FDA	Food and Drug Administration
FOS	Fructo- oligosaccharides
GOS	Galacto-oligosaccharides
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MOS	Mannan-oligosaccharides
MPV	Mean Platelet Volume
PCT	Procalcitonin
RBC	Red Blood Cell
WBC	White Blood Cell
WHO	World Health Organization
XOS	Xylo-oligosaccharides

ACKNOWLEDGEMENTS

My earnest and enormous gratitude goes to the Almighty God. Without His unconditional grace and favour, none of this could have been possible. Next, I would like to express my heartfelt gratitude to my supervisor, Prof. Daniel Boye Okai, for this opportunity. Sir, thank you for your excellent instructions, support, stimulating suggestions, solutions and proof reading. I really appreciate them. To all the lecturers of the Department of Animal Science (KNUST), I say thank you for the services you render to students.

I would also like to thank Dr. John Baah (Agriculture and Agri-Food Canada Research Centre in Lethbridge, Canada) and Dr. Kwame Oppong-Anane (CEO of Oporhu Consultancy Ltd.) for their enormous support in assisting with the acquisition of the Direct-fed microbials (DFM) used in the experiment. To the staff of Basic Environmental Systems and Technology (BEST) Inc., Alberta, Canada, especially Mr. J. G. Watson (CEO), I say thank you very much for providing the DFM and financial resources for this experiment.

My sincere thanks go to Michael Boateng for his companionship, encouragement, support, technical advice on various aspects of the studies and assistance in the statistical analyses. Messrs. L. Gareiba and J. Bentil also deserve thanks for their help with some analytical aspects of the studies. I also cannot overlook Messrs. F. Nixon, Lantei Nathan, and all the workers at the Dept. of Animal Science (KNUST). They were always willing to help in any way possible.

Last, but not least, I would like to thank my parents and siblings for their prayers, financial support and advice and for their moral support especially my sister, Mrs. Ernestina Owusu for her immense financial contributions. God bless you all.

CHAPTER ONE

1.0 INTRODUCTION

The practice of feeding antibiotics at sub-therapeutic doses was very successfully adopted and became an integral part of developing nutritional strategies for all farm livestock. Swine performance is potentially improved by using sub-therapeutic concentrations of antibiotics or chemotherapeutic drugs to increase rate of gain or improve feed conversion efficiency (FDA, 1998). Because of the economic benefit to producers, antimicrobial drugs are used in about 90% of starter feeds, 75% of grower feeds and over 50% of finisher feeds (Cromwell, 1991).

Viola and DeVincent (2006) estimated that about ten million kg of antibiotics were used for livestock production and for companion animals, and nine percent of this (about 900,000 kg) was used for growth promotion purposes. Statistics, compiled in the 1990s (Miller *et al*., 2003), showed that, for an average swine facility, in-feed antibiotics boost daily growth and reduce death rates during production. Similar studies have been conducted in other animal species and resulted in the same conclusions (Samanidou and Evaggelopoulou, 2008). Such antibiotics can cause an increase in feed conversion efficiency of 1-8% (Jongbloed, 1998). This increase depends, among other factors, on the hygiene level on the farm, the age of the animal and the influence of other feed additives.

The primary effects associated with the inclusion of antimicrobial feed additives are prevention of digestive disturbances, improved feed utilization, and improved animal performance. Secondary effects include reduced nutrient wastage, diminished environmental impact and reduced production costs. The greatest benefit of antibiotics when used as a means of improving feed efficiency and promoting growth occurs in the nursery period and therefore, it is not surprising that surveys of drug use

on pig farms have revealed that almost all pigs are fed antibiotics during the immediate post-weaning period (Dunlop *et al*, 1998).

However, there is growing pressure on the livestock industry to reduce the use of antibiotics or for a ban on their use. This means that the practice of using antibiotics at low levels in the feed for growth promotion is particularly under scrutiny. The continued feeding of antibiotics at sub-therapeutic levels has created concerns about the extent to which usage increases the possibilities of antibiotic residue (in meat**,** vegetables, manure, soil, air and surface waters), the development of drug-resistant bacteria, and a reduction in the ability to cure bacterial diseases in humans (Jensen, 1998; Kumar *et al*., 2005). Indeed, there is evidence that antibiotic resistance genes can be and are transmitted from animal to human microbiota (Greko, 2001). Monitoring and identifying resistance mechanisms and their dissemination into the food chain were recently reviewed by Roe and Pillai (2003). The WHO (1997) published a report on the medical impact of the use of antimicrobials in food animals and suggested a link between the two on an epidemiological basis. This report (WHO, 1997) recommends, on precautionary grounds, that national governments adopt a proactive approach to reduce or ban the need for antimicrobial use in animals and establish surveillance of antimicrobial usage and resistance. The recommendation is precautionary, and is based on the potential for a reservoir in food animals of an antibiotic resistant bacteria population (primarily enterococci) that could be transferred to humans.

Increased awareness of the potential problems associated with the use of antibiotics has stimulated research efforts to identify alternatives to their use as feed additives.

One such option that has received increasing attention is the use of direct-fed microbials (DFM), also known as probiotics. Martin and Nesbit (1992) stated that the U.S. Food and Drug Administration defines a direct-fed microbial (DFM) as a source of live (viable), naturally occurring non-pathogenic organisms that have beneficial effects in preventing or treating several enteric disease conditions and currently requires manufacturers to use the term 'direct-fed microbial' (DFM) instead of probiotics. According to FAO/WHO (2001)**,** probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. This definition covers all three categories of probiotics: yeast, fungi, and bacteria. The detailed mechanism of action of DFM remains unknown, but it is believed that they act by modifying the ecology of the intestinal microflora. Unlike antibiotics, DFM introduce live beneficial bacteria into the intestinal tract and neither generates antimicrobial resistance nor produce toxic compounds in carcasses (Fuller, 1989).

Supplementation with DFM have been suggested to benefit the host animal by stimulating appetite, improving both intestinal microbial population balance (Fuller, 1989) and digestion (Collins and Gibson, 1999). Furthermore, studies have also suggested a role for probiotics in stimulating the immune system (Collins and Gibson, 1999**;** Perdigon *et al*., 1990). A fairly recent study suggests that a specific strain of probiotics, *Bacillus coagulans* GBI-30, PTA-6086, increases the body's immune response to the flu virus (Huffnagle, 2009). According to Dawson (1993), DFM have been shown to increase daily gain and feed efficiency in feedlot cattle, enhance milk production in dairy cows and improve health and performance of young calves. Several strains of bacteria have been used efficiently to produce DFM, but the most common are *Lactobacilli, Bacillus, Bifidobacteria* and *Streptococci* species. Yeasts

may also be used to manipulate the conditions within the gut and both *Saccharomyces cerevisae* and *Aspergillus sp.* have been most commonly included in the diets of monogastric animals. Microbial strains need to survive the acidic conditions in the stomach and the bile salts in the duodenum in order to exert their beneficial effects in the gut. Therefore, bile tolerance is considered one of the most important properties of direct-fed microorganisms, because it allows them to survive and to colonize the gastrointestinal tract (Gómez-Zavaglia. *et al*., 2002).

Many DFM products are commercially available for livestock production. The one used in this study was developed by Basic Environmental Systems and Technology (BEST) Inc., in Alberta, Canada. There is a lack of information on the effects of DFM on pigs in the tropics, however recently a study was conducted using a DFM product containing *Lactobacillus sp, Bacillus sp* and *Saccharomyces cerevisiae* and Mazorite (a naturally mined mineral product that contains a broad spectrum of metabolically active clay minerals and 74 trace elements) to determine the growth performance and carcass characteristics of growing pigs (Okai, 2008). His results suggest that the addition of DFM and Mazorite to the diets of growing pigs did not seem to improve the growth performance and feed efficiency significantly. He, however, recommended that further studies should be undertaken over an extended period of time so as to confirm or otherwise the effects of DFM on pigs in the tropics. This study which covered a period of twenty-one weeks sought to provide further evidence on the effects of the same RE-3, a DFM preparation on the growth performance, blood profile and carcass characteristics of pigs in the tropics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FEED ADDITIVES IN SWINE DIETS

Feed additives are feed ingredients of non-nutritive nature which stimulate growth or other types of performance or improve the efficiency of feed utilization or that may be beneficial in some manner to the health or metabolism of the animal (Kellems and Church, 2002). Lewis (2002) also defined feed additives as compounds that are added directly to a feed to improve flavour, odour and appearance, to preserve or extend its useful life and to enhance its natural properties. Feed additives provide a means by which dietary deficiencies can be addressed and this benefits not only the nutrition and thus the growth rate of pigs but also their health. They elicit a response independent of contributions to the pig's energy, amino acid, mineral and vitamin requirements. Typically, feed additives are added to pig diets in small amounts and have been used extensively in swine diets since the 1950s (Gillespie, 1998). Most swine producers use them because of their demonstrated ability to increase growth rate, improve feed utilization, and reduce mortality and morbidity from clinical and subclinical infections (Tisch, 2006).

2.1.1 BENEFITS OF FEED ADDITIVES

The most effective use of feed additives are in the diets of weanling and young growing pigs but responses are also obtained in finisher pigs and breeding swine. Zimmerman (1986) summarized the data from 239 separate experiments and reported that average improvement response to feed additives in starter pigs was 15% for growth rate and 6% for feed efficiency. In older growing-finishing pigs, the

improvement was 4% for growth rate and 2% for feed efficiency. It is believed that weanling and starter pigs are more susceptible to stress and sub-clinical diseases and consequently show a greater response to growth-promoting antimicrobial products. Studies have also indicated that both starter and finisher pigs have a greater response to antimicrobials under farm conditions than at swine research facilities, possibly because the disease, sanitation and housing stresses are typically greater in commercial farms than in research facilities. The use of feed additives for diet manipulation reduces N and P content in swine manure and minimizes the negative effects of odour and other gaseous emissions from swine waste (Cromwell *et al*., 1998). Research has suggested that the inclusion of "metabolic modifiers" (i.e. feed additives) in the diet for the last five weeks of finishing increased the fat-free lean by up to 3.8%, increased loin eye area by 5.0%, reduced rib backfat by 18% and increased protein production by 2.4% without adversely affecting meat quality traits (Herr, *et al*., 2000).

However, the use of feed additives has suffered restrictions due to its general misuse and claims that there could be development of resistant strains of microorganisms that might be pathogenic to human and work against human antimicrobial drugs (Kellems and Church, 2002).

2.1.2 TYPES OF FEED ADDITIVES

There are several methods for describing the types of feed additives available. Only three of these would be discussed here: According to the European Food Safety Authority (EFSA, 2003), feed additives are categorized based on their action on the feed and in the animal. They described them as:

i. Technological additives: This category refers to a group of additives which influences the technological aspects of the feed. It does not directly manipulate the nutritional value of the feed but may do so indirectly by improving its handling or hygienic characteristics. For instance, an organic acid for preservation of feed.

ii. Sensory additives: This group improves the palatability (i.e. voluntary intake) of a diet by stimulating appetite, usually through the effect these products have on the flavour or colour of the diet. For example, a vanilla extract may well encourage piglets to eat a ration.

iii. Nutrient additives: Such additives provide specific nutrient(s) required by the animal for optimal growth. Examples are vitamins, amino acids or trace minerals. In most cases, such additives are simply concentrated forms of nutrients supplied in natural ingredients in the diet.

iv. Zootechnical additives: These additives improve the nutrient status of the animal, not by providing specific nutrients, but by enabling more efficient use of the nutrients present in the diet. Examples are enzymes and direct fed microbial products, both of which enhance the conditions of the intestinal tract, thus enabling more effective nutrient extraction from the diet. In this respect they are often referred to as "pro-nutrients", i.e. products which improve the nutritional value of a diet without necessarily providing nutrients directly. Other additives in this category are used for the environmental benefits that they provide to the animal husbandry and others are targeted for specific physiological functions.

v. Coccidiostats: These are used to control enteric health of poultry through direct effects on the parasitic organism concerned.

Dritz *et al*. (1997) on the other hand, classified the additives available for swine producers as:

i. Growth-promoting minerals
ii. Enzymes
iii. Organic acids
iv. Antimicrobials which include: antibiotics, chemotherapeutics and anthelmintics or dewormers
v. Direct-fed microbials or Probiotics

Earlier Banerjee (1988) had classified feed additives into two main types. These are:

i. Nutritive feed additives: these are nutrients and therefore have nutritive value necessary for proper development of the animal. Examples are vitamins, minerals, amino acids, etc.
ii. Non-nutritive feed additives: these do not supply nutrients, but rather stimulate growth and improve efficiency of feed conversion, under certain conditions, when added to the diet at low concentrations. For instance; antimicrobials (antibiotics, chemotherapeutics, anthelmintics etc) and coccidiostats.

The area that will be covered in this thesis has considerable relevance to non-nutritive feed additives and therefore subsequent discussions will concentrate on this type of additive.

2.1.2.1 NON-NUTRITIVE FEED ADDITIVES

These feed additives are often included in swine diets and of these; the antimicrobial agents are the additives most commonly used. Antimicrobials are defined as 'drugs' that kill or inhibit the growth of microorganisms such as bacteria, fungi or protozoas, as well as destroying viruses. The primary effects associated with the inclusion of an

antimicrobial feed additive are: prevention of digestive disturbances, improved feed utilisation and improved animal performance. Thus, their usage levels, allowable combinations and periods of withdrawal prior to slaughter are regulated by the FDA and are published annually in the Feed Additive Compendium (2004). In addition, certain other additives are sometimes included in swine diets. The Association of American Feed Control Officials (1998) has established guidelines for the use of many of these products in animal feeds. Of all the antimicrobials used in swine production, antibiotics are used mostly as growth promoters.

2.1.2.2 ANTIBIOTICS

The term antibiotic means "against life" or "destructive to life." Antibiotics are compounds of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms. Kellems and Church (2002) defined antibiotics as compounds produced by micro-organisms which have properties of inhibiting the growth or metabolism of some micro-organisms. Nearly all antibiotics are produced by bacteria or molds. Many microorganisms produce antibiotics that inhibit or kill other organisms, a process called *antibiosis.* The discovery and development of antibiotics, one of the major scientific and medical advances of the 20th century, has led to dramatic improvements in treating infectious diseases and has significantly increased food-animal production. A large number of pharmaceuticals are counted among the antibiotics. They can be divided into different chemical groups, eg: tetracyclines, macrolides, penicillins, aminoglycosides, sulfonamides or the fluoroquinolones.

The first antibiotics to be tested on animals in the late 1930's were sulfonamides. They were however considered to be unfavourable after causing agranulocytosis and

reduced growth rate in rats (Visek, 1978). Later, researchers found increased growth and reduced mortality after supplementing rat diets with sulfonamides and essential vitamins (Visek, 1978). Antibiotics were readily adopted into feeding programmes by livestock producers after their growth enhancing capabilities were reported in the early 1950's. However, the FDA first approved them as feed additives for farm animals in 1951. Since then a variety of antibiotics has been used sub-therapeutically for most livestock produced. Cromwell (1991) estimated that about three thousand tonnes of antibiotics were used in livestock feeds in the United States alone. The most current estimate is around eight thousand tonnes (Cromwell, 2002). It has estimated that about ten thousand tonnes of antibiotics were used for livestock production and for companion animals, and nine percent of this (about 900 tonnes) was used for growth promotion purposes (Viola and DeVincent, 2006). Typically, they are administered to livestock through the feed, water or by injection.

Antibiotics can result in an increased feed efficiency of 1%- 8% (Jongbloed, 1998). The extent of the increase depends, among other factors, on the hygiene level on the farm, the age of the animal and the influence of other feed additives. The primary effects associated with the inclusion of antimicrobial feed additives are: prevention of digestive disturbances, improved feed utilization and improved animal performance. Secondary effects include reduced nutrient wastage, diminished environmental impact and reduced production costs.

Antibiotics are used largely for three purposes in animals: therapeutic (more than 200 g/ton of feed for 14 days or less) use i.e. to treat sick animals; prophylactic use i.e. to prevent infection in animals; and as growth promoters (less than 200 g/ton and for more than 14 days) to improve feed utilization and production.

In general, therapeutic treatment involves treatment of individual animals over a short period with doses of antibiotic exceeding the minimal inhibitory concentration of the known or suspected pathogen. Sometimes, with intensively farmed animals, therapeutic treatment is delivered by feed or drinking water; however, this treatment can be of doubtful efficacy in some situations, as sick animals often do not drink or eat.

Prophylactic treatment involves moderate to high doses of antibiotics, often given in feed or water for a defined period to a group of animals. Antibiotics used as growth promoters tend to be given in feed at sub-therapeutic levels over extended periods to entire herds and flocks. Viola and DeVincent (2006) stated that antibiotics can be used at therapeutic levels to treat disease, intermediate (prophylaxis) levels to prevent disease, or "metaphylaxis" levels to treat disease in sick animals while preventing disease in non-infected animals.

Most notably however, in swine production antibiotics are used at sub-therapeutic levels to enhance growth performance. Early work by Cunha *et al*. (1950) showing the growth-promoting effect of using antibiotics in pig diets resulted in immediate adoption of this practice in the swine industry. The USDA (2002) estimates that antibiotics are used in feed for 80% of pigs from weaning to market, 51% of sow/gilt feed, and 28% of boar feed in the United States. Cromwell (2002) summarized 13 experiments and reported that piglet survival and the weaning weights of pigs were increased when antibiotics were included in farrowing and lactation rations. The antibiotics also allow for more efficient intestinal growth and may reduce growth-depressing microbial metabolites, subclinical infections and competition for nutrients by microorganisms through modification of the gut microflora and therefore, improve growth rate (Anderson *et al*., 1999).

2.1.2.2.1 MODE OF ACTION OF ANTIBIOTICS

The mode of action by which antibiotics improve growth rate and feed efficiency is not well understood, but generally is attributed to metabolic, nutritional and disease-control effects (Cromwell, 2001).

The metabolic effect suggests that antibiotics directly affect the rate or pattern of the metabolic processes in the animal, such as influencing metabolic rate, nitrogen or water excretion, rate of nutrient oxidation, etc. Antibiotics can alter the requirement for energy, fat, protein, water and fat-soluble vitamins and minerals. Growth promotion by antibiotics is generally greater for diets based on sucrose compared to starch diets (Visek, 1978).

The nutritional effect has a substantial amount of research support. Certain microbes that inhabit the intestinal tract produce vitamins and amino acids which are essential to animals, while other microbes compete with the host animal for essential nutrients. Bacterial use of glucose may decrease the net energy available to pigs by as much as 6% (Gaskins *et al.*, 2002). Shifts in bacterial populations associated with the feeding of antibiotics could account for a greater availability of nutrients for the host animal. Antibiotic feeding has also been shown to reduce the thickness of the gut wall, resulting in a potential for greater absorption of nutrients. The gut wall thickening is thought to be caused by bacteria that damage or produce toxins that, in turn, damage intestinal tissue. The increased ammonia production that occurs in the gut when non-antibiotic diets are fed is thought to be a major contributing factor to the increased gut wall thickness (Visek, 1978).

The disease-control effect is the most widely accepted explanation for the growth response to antibiotics. This mechanism implies that antibiotics suppress those organisms in the intestinal tract that are responsible for subclinical or nonspecific

diseases, thereby allowing pigs to perform up to their maximum genetic potential. Cromwell (2001) found, in his review, that antibiotics are most effective at improving growth of slow-growing and unthrifty pigs possibly due to suppression of bacteria causing subclinical diseases. The growth response to antibiotics is influenced by several factors: the stage of growth of the pig, the cleanliness of the environment, the disease level in the herd and the level and type of antibiotics. Young pigs have lower levels of immunity and are more susceptible to disease-causing organisms in their environment; therefore, they respond to antibiotics more than do older pigs. Responses to antibiotics tend to be greater under conditions of poor sanitation, poor management and high disease incidence. Again, this can be explained by the greater growth depression from subclinical diseases in the poorer environment, which is partially alleviated by the feeding of antibiotics.

It is likely that all these proposed modes of action work in concert with each other to produce the overall growth-promoting effect of antibiotics. However, antibiotics usage at sub-therapeutic levels have come under much scrutiny, as it has been shown to contribute to the increased prevalence of antibiotic residues (in meat**,** vegetables, manure, soil, air and surface waters) and the development of antibiotic-resistant bacteria of human significance (Kumar *et al*., 2005).

2.1.2.2.2 GROWTH PROMOTION EFFECTS OF ANTIBIOTICS

The phrase "antibiotic growth promoter" is used to depict any drug that kills or hinders bacteria and is administered at a low, sub-therapeutic dose. Antibiotics for growth promotion have become common with the escalation of livestock farming. Antibiotics used for growth-promoting purposes constitute a large proportion of the total antibiotic usage, but the scale of its residue in the environment is difficult to

estimate since there is little published information on the overall quantities of antibiotics used in animals.

Cunha *et al*. (1950) was the first to demonstrate that feeding an antibiotic (aureomycin) to pigs improved daily gain and feed efficiency compared to pigs receiving a control diet. Since that time, feeding swine with sub-therapeutic levels of antibiotics has been espoused. According to the National Office of Animal Health (NOAH, 2001), antibiotic growth promoters are used to "help growing animals digest their food more efficiently, get maximum benefit from it and allow them to develop into strong and healthy individuals. Antibiotics at sub-therapeutic dosages have been shown to reduce the incidence or severity of swine dysentery, porcine intestinal adenomatosis, porcine haemorrhagic enteropathy and *Clostridium perfringens* infections. Most scientists suppose that these effects on pathogens are the principal or only relevant effect of the antibiotics affecting growth promotion.

Currently, 18 antimicrobials are approved for use in swine feeds by the FDA. The lists of the approved antimicrobials and their recommended growth promoting levels are shown in Table 1. Research shows that a wide variety of antimicrobials have growth promoting ability. Zinc-bacitracin, chlortetracycline, sulfamethazine, sulfathiozole, penicillin, tylosin, oxytetracycline, neomycin, and tilmicosin have all been shown to improve growth performance of pigs (Weber *et al*., 2001).

2.1.2.2.3 ANTIBIOTIC RESIDUES

A residue is a substance, or its metabolite, that remains in any body tissue after absorption. The original substance may have been a feed or water additive, an injectable or topical treatment or an accidental contaminant.

Table 1. Antimicrobials Currently Approved by FDA for Use in Swine Diets*

Antimicrobial	Class	Growth-Promoting Level	Trade Name
Antibiotics			
Apramycin	Aminoglycoside	150 g/ton	Apralan
Bacitracin Methylene Disalicylate	Bacitracin	45-90 g/ton	BMD
Bacitracin zinc	Bacitracin	10-50 g/ton	Albac
Bambermycin	Bambermycin	2-4 g/ton	Flavomycin
Chlortetracycline	Tetracycline	10-50 g/ton	Aureomycin
Lincomycin	Lincosamide	20 g/ton	Lincomix
Neomycin	Aminoglycoside	20 g/ton	Neomix
Oxytetracycline	Tetracycline	10-50 g/ton	Terramycin
Penicillin	B-lactam	10-50 g/ton	CSP-250, CSP-500
Tiamulin	Diterpene	10 g/ton	Tiamutin
Tilmicosin	Macrolide	20-100 g/ton (starter) 20-40 g/ton (grower) 10-20 g/ton (finisher)	Pulmotil 90
Tylosin	Macrolide	20-100 g/ton (starter) 20-40 g/ton (grower) 10-20 g/ton (finisher)	Tylan
Virginiamycin	Streptogram	5-10 g/ton	Stafac
Chemotherapeutics			
Arsanilic acid	Arsenical	10-30 g/ton	Pro-Gen 20%
Carbadox	Quinoxaline	10-25 g/ton	Mecadox
Roxarsone	Arsenical	22.7-34.1 g/ton	3-Nitro
Sulfamethazine	Sulfonamide	100 g/ton	Tylan 40, Sulfa-G
Sulfathiazole	Sulfonamide	100 g/ton in combination with chlortetracyclie	CSP-250, CSP-500

* Source: Feed Additive Compendium, 2004.

Some substances may be cleared from body tissues a few hours after absorption, others several months and some may never be entirely cleared. Until very recently, controls on antibiotic use in animals focused almost exclusively on the control of residues in the tissues of treated animals. Animals excrete a significant fraction of the antibiotics they consume. These wastes and the antibiotics and resistant bacteria in

them, are typically transferred to the environment (soil/manure, water bodies, air, vegetables etc). Kumar *et al*. (2005) reported the presence of antibiotic residues in vegetables. The most prevalent antibiotic residues found in the environment have been some of the macrolide and sulfonamide groups, whereas fluoroquinolones, tetracyclines or penicillins have only been found in some cases and at generally low concentrations. These antibiotics in the environment can promote development of resistance in bacteria naturally present in the soil, water, crops etc. In USA, an estimated 6.12 million kg of antibiotics are excreted annually in animal wastes as a result of using antibiotic feed additives. This is nearly half of the total amount of antibiotics added to feeds. Concerns about residues revolve around allergic reactions and the possible adverse effects on the flora of the human gastrointestinal tract.

2.1.2.2.4 DEVELOPMENT OF ANTIBIOTIC RESISTANCE

The term "resistant" came into use to describe classes of bacteria against which an antibiotic was used effectively for some time but later became ineffective. Bacteria are incredibly adaptable organisms because of their extremely short generation time (as little as 15 to 20 minutes for some species under ideal conditions) and their tendency for sharing genetic information even among different species of bacteria (Newman and Scheuren-Portocarrero, 2005).

When antibiotic drugs are brought into contact with plying susceptible microorganisms, the organisms are generally inhibited from plying further or are killed. When the susceptible organisms constitute a portion of the total microbial flora that is exposed to the drugs, the elimination of the susceptible organisms is generally followed by some degree of compensatory multiplication of the more resistant or non-susceptible strains. They consequently pass their resistance genes on to their offspring

and habitually, to other species of bacteria. Both medical and veterinary uses of antibiotics have resulted in the appearance of resistant strains of bacteria. There are two processes by which bacteria can express resistance to an antibiotic, i.e. either intrinsically or by acquired resistance.

Intrinsic resistance to an antibiotic, which occurs naturally, is a specific property of each genus or species of bacteria. For instance, Enterococci species have a broad range of intrinsic resistance to antibiotics, including semi-synthetic penicillins, aminoglycosides, lincosamides, streptogrammins, cephalasporins, and quinolones (Klare *et al*., 2003). Gram-negative bacteria, such as *E. coli*, are intrinsically resistant to glycopeptides because their outer membrane is impermeable to the large molecule. It is imperative to note that antibiotics are naturally produced by many organisms and antibiotic resistance would occur even without medicinal use of antibiotics (Mathew *et al*., 2003).

Acquired resistance is a consequence of modification of the genome of the microorganism, with the principal mechanism being the mutation of a gene into a resistance gene. This usually occurs at a fairly low rate. Acquired resistance can also occur by incorporation of genes from a donor bacterium into the acceptor by conjugation, transformation, or transduction, which is known as horizontal gene transfer (Catry *et al*., 2003). Transduction occurs when DNA is transferred between two closely related bacteria, while transformation is a process by which free DNA from the environment is taken into the bacteria (Newman and Scheuren-Portocarrero, 2005). Catry *et al*. (2003) reported that transduction is rare and the occurrence of transformation is possibly underestimated. The gastrointestinal tract of swine is a primary reservoir of zoonotic bacteria and provides an exceptional environment for the exchange of genetic information (Newman and Scheuren-Portocarrero, 2005). The

most important mechanism for horizontal gene transfer is conjugation, which involves the spread of mobile genetic elements such as plasmids (Catry *et al*., 2003). Conjugation is the major mechanism by which gram-negative bacteria transfer DNA and has been shown to occur between gram-negative and gram-positive bacteria (Newman and Scheuren-Portacarrero, 2005). Schnappinger and Hillen (1996) reported that tetracyclines can promote the frequency of conjugation. Plasmid transfer has also been shown to occur between pathogenic bacteria from different species origin (porcine, bovine, fish etc) to humans (Newman and Scheuren-Portacarrero, 2005). Even at non-inhibitory concentrations, antibiotics are able to change gene expression without killing the bacteria (Hoffman, *et al*., 2007). Once a resistance gene has been established, bacteria use different mechanisms to induce resistance to an antibiotic. Resistance mechanisms are related to the mode of action of the antibiotic on the bacteria, but bacteria can possess more than one resistance mechanism (Catry *et al*., 2003). Antibiotic-resistant strains of bacteria, including *Salmonella spp., E. coli* and *Campylobacter spp.,* have been isolated from farm animals in many countries (Aarestrup *et al*., 1997).

2.1.2.2.5 EFFECTS OF RESISTANT PATHOGENS IN SWINE PRODUCTION

Pigs are usually reared in confinement much like poultry, and both pneumonia and diarrhoea are common problems. Most pigs are given antibiotics through their feed for disease prevention or growth promotion. Research indicates that use of antibiotics in swine decreases pathogen load (Bach-Knudsen, 2001). However, research has demonstrated that antibiotic-resistant pathogenic bacteria do exist in swine facilities. Gebreyes *et al.* (2000) found many drug-resistant *Salmonella* isolates from pig faeces. Payot *et al*. (2004) reported that *Campylobacter coli* isolated from market weight pigs

were resistant to ampicillin and tetracycline and 37% of isolates were drug resistant. Antibiotic-resistant pathogenic bacteria in swine, especially at market age, can be a serious threat to human health. Most reports on antibiotic resistance in isolates from swine involve *Enterococcus spp.*

The Enterococci are a group of gram-positive cocci that are part of the normal resident flora of both swine and humans (Melhus and Tjernberg, 1996). They are generally not considered virulent; however, their intrinsic resistance to many antibiotics (including cephalosporins, penicillin, and aminoglycosides) has made them important opportunistic pathogens. Although the Enterococci are opportunistic pathogens, they are a frequent cause of bacteremia and endocarditis, which are all difficult to treat due to resistance.

Staphylococci are another species of gram positive cocci that are often associated with infections and antibiotic resistance in swine. The most important pathogen of the staphylococci species is *Staphylococcus aureus* which is commonly found on the skin and in the nasal passages of pigs. *S. aureus* infections cause impetigo in newborns, pneumonia, endocarditis and septicemia (Le Loir *et al.*, 2003). Currently, more than 90% of *S. aureus* and between 50% and 70% of coagulase-negative staphylococci are resistant to most antibiotics.

Streptococci are spherical gram-positive bacteria that are also normally linked with contagions and antibiotic resistance in swine. The three main pathogens of concern are *Streptococcus pyogenes, Streptococcus agalactiae* and *Streptococcus pneumoniae. Streptococcus pneumoniae* or pneumococcus is the leading cause of both meningitis and septicemia. In addition, *S. pneumoniae* can cause pneumonia, sinusitis, otitis media and other respiratory infections. By the time penicillin came into use, both *S. pneumoniae* and *S. pyogenes* were commonly resistant to the sulfonamides.

Escherichia coli are very common gram-negative (rod) bacteria normally found in the intestinal tract of pigs. It is the predominant isolate in the faecal flora of most pigs and has the ability to transfer resistant genes to other bacteria (Anderson *et al*., 1999). Mathew *et al.* (2003) have shown that weanling pigs exposed to antibiotics had an increased prevalence of antibiotic resistant *E. coli* compared to pigs that did not receive antibiotics and reported that all *E. coli* isolated from swine faeces were resistant to at least 8 antibiotics, 86% were resistant to 11 antibiotics and 2% were resistant to 16 antibiotics, with the most resistance occurring against tetracycline, sulfamethoxazole, ampicillin and streptomycin.

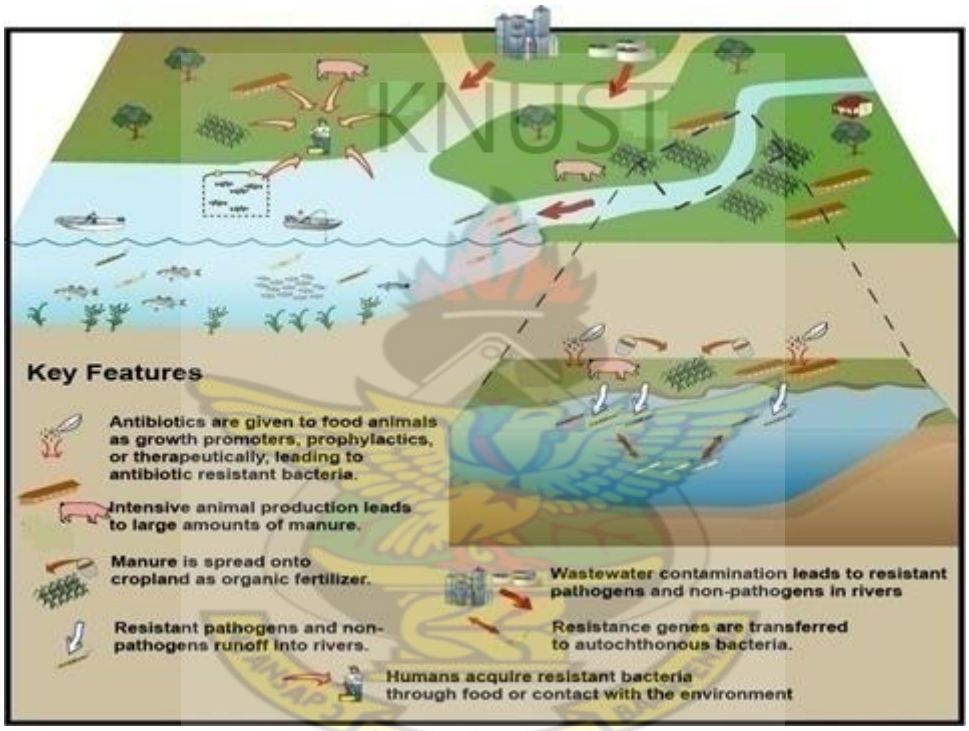
There is evidence that although resistant microorganisms existed in nature before the use of antibiotics, such microorganisms were mostly absent from human flora (Hughes and Datta, 1983).

2.1.2.2.6 THE SWINE-ENVIRONMENT-HUMAN WEB OF RESISTANCE

The use of antibiotics could contribute to increased antimicrobial resistance in human pathogens through several routes, including human consumption of antibiotic residues in animal products, human exposure to antibiotics and resistant microorganisms during animal care and contamination of ground and surface waters, soils and crops by wastes containing antibiotics and resistant microorganisms. As illustrated in Figure 1, agricultural antibiotic use leads to increased resistance in the environment since resistant organisms and antibiotic residues are excreted as waste that are frequently spread onto farmland as organic fertilizer. Faecal bacteria survive long periods in the environment and spread through runoff into groundwater, rivers, and marine ecosystems. Eventually, it is the prospect of being unable to treat human disease with antibiotics that is most disturbing. Obviously, the use of antibiotics leads to increased

antibiotic resistance in swine, humans and the environment. Resistant genes can spread via horizontal gene transfer in practically every environment. There is sufficient evidence to support the hypothesis that resistant organisms and resistant genes spread from swine to humans and to the environment and vice versa. Indeed, there is evidence that antibiotic resistant genes can be and are transmitted from animal to human microbiota (Greko, 2001).

Figure 1. Swine-Environment-Human Web of Resistance: Conceptual Diagram



Source: Center for Environmental Science, University of Maryland (2005). Symbols for diagrams courtesy of the Integration and Application Network (ian.umces.edu/symbols)

Gassner and Wuethrich (1994) demonstrated the presence of chloramphenicol metabolites in meat products and concluded that a link with the presence of these antibiotic residues in meat and the occurrence of aplastic anaemia in humans cannot be ruled out.

Resistant bacteria which are human pathogens may cause diseases that are difficult to treat; even if the resistant bacteria are not human pathogens, they may still be

dangerous because they can transfer their antibiotic resistance genes to other bacteria that are pathogenic (Khachatourians, 1998). Also, much of the evidence comes from the presence of similar gene sequences in bacteria isolated from swine and humans and from examination of antibiotic-resistant pathogens isolated from diseased swine that were traced to humans.

In a study of human isolates of *Bacteroides* and isolates of *Prevotella* (normally found in the gastrointestinal tract of livestock) from different areas, sequences of the tetracycline resistance gene *'tetQ'* were almost identical. In a CDC investigation of salmonellosis outbreaks from 1971 to 1983, researchers found that of 38 outbreaks, where the source could be identified, food animals were the source of 69% of the antibiotic resistant strains and 46% of susceptible strains (Holmberg *et al*., 1984).

As a result of concerns of antibiotic residues and bacterial resistance to antibiotics (Cromwell, 2002), their use has been restricted, giving way for other feed additives to be used.

2.2 ALTERNATIVES TO THE USE OF ANTIBIOTICS

Many alternatives to antibiotic growth-promoters have been proposed and due to the possible restrictions on the use of sub-therapeutic antibiotics in the future, these alternatives will be needed. A systematic approach involving nutrition, immunology, and management will be required to determine a cost-effective method to maximize pork production without the use of antibiotic growth promoters (Adijiri-Awere and van Lunen, 2005).

The alternative strategies that are available as replacement products to antibiotics include:

- Organic acids (Acidifiers)

- Prebiotics (Oligosaccharides)
- Enzymes
- Growth-promoting minerals
- Synbiotics
- Direct-fed Microbials/Probiotics

2.2.1 ORGANIC ACIDS (ACIDIFIERS)

Organic acids contain one to seven carbon atoms and are commonly referred to as acidifiers. They are widely distributed in plants and animals and are also produced during microbial fermentation. These acids and their salts are often used as food preservatives and since they are easy to handle, can be used to acidify feed. Some of the most used acids and their salts are: formic, acetic, propionic, butyrate, lactic, fumaric, Ca-formate, Ca-propionate, K-diformate and Na-benzoate (Mroz, 2003). Organic acids and salts appear to be potential alternatives to prophylactic in-feed antibiotics for improving the performance of weaned piglets, fattening pigs and reproductive sows. Weaned piglets are physiologically immature and may not produce enough hydrochloric acid to keep stomach pH at an optimum of approximately 3.5. At this pH, digestion of proteins and populations of beneficial bacteria (lactobacilli) are maximized and harmful bacteria are inhibited. Diets fed to young pigs often have a high buffering capacity, which can further reduce stomach acidity. Therefore, organic acids added to feed can have a beneficial effect in maintaining a low pH. Siljander-Rasi *et al*. (1998) demonstrated the improved feed conversion ratio and growth-promoting effects of formates, citric acid and formic acid and indicated that the effect was greater during growth of young pigs than during the finishing phase of growth.

Eisemann and Heugten (2007) observed 3.5% improvement in efficiency of gain when formic acid was fed to grower-finisher pigs. Organic acids may be used singly but blends of organic acids are usually more potent than single organic acids (Namkung *et al*., 2004). The mode of action of particular organic acids and salts is not uniform, although a consensus seems to be achieved on the following:

i. That undissociated forms diffuse across cell membranes of pathogens, destroying their cytoplasm or inhibiting growth (inactivation of bacterial decarboxylases and catalases);
ii. Intestinal dissociation liberates H+ ions serving as a pH barrier against pathogen colonisation on the brush border;
iii. Reduced gastric pH in the stomach;
iv. Gastric hydrolysis liberates H+ ions activating pepsinogen and inhibiting bacterial growth (bactericidal/bacteriostatic effects);
v. Precursors for synthesis of non-essential amino acids, DNA and higher lipids required for intestinal growth;
vi. Increased blood flow and hypocholesterolemic effect (Mroz, 2005).

Organic acids at 0.45 kg per ton have been approved for controlling molds in feeds; higher concentrations (>2.7 kg/ton) reduce the pH of feed to 5.0 and help control *Salmonella* and other enteric pathogens. Two problems may occur at higher organic acid levels:

i. Palatability may be decreased, leading to feed refusal (Partanen and Mroz, 1999) and
ii. Acidic feed is corrosive to cement and galvanized steel in swine housing. In order to minimize these effects, the natural buffering capacity of feeds (related

to mineral and protein content) should be evaluated to determine the minimum effective amount of acid to use (Best, 2000).

2.2.2 PREBIOTICS (OLIGOSACCHARIDES)

Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host animal by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (McKean, 2004). Patterson and Burkholder (2003) also defined it as “non-digestive, yet fermentable sugars used to promote the proliferation of beneficial bacterial population in the gastrointestinal tract”. Therefore prebiotics are food ingredients neither hydrolysed nor absorbed in the upper intestine and are therefore available in the caecum-colon where they are fermented by a limited number of "indigenous" bacteria. By the 1980’s, the possible potential effects of prebiotics in animal feeds was already recognized. Since then, the interest in the use of prebiotics in animal feed and pet food has resulted in a high research activity.

The use of prebiotics in diets for farm animals and pets has been documented by researchers (Santos *et al.* 2005; Patterson and Burkholder, 2003). The main focus of work on prebiotics has been to reduce the negative effects of bacterial diseases of the pig’s colon, such as swine dysentery, colonic spirochetosis and salmonellosis, by indirectly encouraging the growth of bacteria considered to be associated with a healthy gut, primarily *Lactobacilli* and *Bifidobacteria spp*. Generally, prebiotics have also led to a change in metabolic activity of the intestinal flora causing an increase in carbohydrate fermentation and a decrease in protein degradation and fermentation.

Pig health and performance are improved with inclusion of prebiotics in diets of piglets since prebiotics increase the release of cytokines (which coordinates the action of the immune system). Santos *et al.* (2005) recently observed that a high

concentration of plant cell wall polysaccharides in wheat-based turkey diets shifted the intestinal microflora of poults to a healthier state and decreased *Salmonella spp.* population in the caecum. Several important commensal bacteria that are present in a "healthy gut" cannot be cultured, so they cannot be used in commercial probiotic products. However, dietary supplementation of prebiotics has been shown to stimulate these unculturable bacteria in pigs (Konstantinov *et al.*, 2003). Moreover, they have the advantage of being more stable to the heat and pressure incurred during feed processing. Also they have an economical advantage because some of the best prebiotics are derived from inexpensive food processing by-products (Playne and Crittenden, 1996).

To be classified as a prebiotic, a food ingredient must be:

- Neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract.
- A selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon, thus stimulating the bacteria to grow or become metabolically activated, or both; and
- Able as a consequence to alter the colonic micro-flora towards a healthier composition (Collins and Gibson, 1999).

Sources of natural prebiotics are the seeds of some Leguminosae (soya, peas, broad beans, lupins), yeasts in whose walls, MOS are widely represented, which are well-known for their prebiotic activity. Arabinoxylans, glycosylsucrose, inulin, AOS, FOS, raffinose, GOS, stachyose, MOS, XOS, isomaltose, lactosucrose, lactulose and lactose are the main prebiotics used (Patterson and Burkholder, 2003). Mul and Perry (1994) found that the inclusion of FOS improved the growth rate of weanling pigs by 5.1% and reduced FCR by 2%. Notwithstanding the above-mentioned benefits, research conducted by Mikkelsen *et al.* (2003), intimated that prebiotics remains controversial,

and added that no firm recommendations can be made regarding the practical application of such technology.

2.2.3 ENZYMES

Enzymes are organic catalysts which affect and speed the rate of chemical reaction without appearing in the final product (McDonald *et al.,* 1992). According to Bagyaraj and Rangaswani (2001), they are organic catalysts produced by biological systems which are also capable of acting independently of the living cell. They are specific for their substrates similar to a key being specific for a particular lock.

The use of exogenous enzymes in animal nutrition is a current practice in non-ruminant species, mainly swine and poultry. The primary objective is to increase nutrient digestibility, either by supplementation of the endogenous enzymatic activity (α-amylase and protease in piglets), or those activities not existing internally (xylanase, β-glucanase, α-glucosidase and phytase). The enzymatic supplementation in swine is centred on the overriding of anti-nutritional factors in the diet and in the improvement of protein digestibility, especially that of vegetable origin. Improving the digestibility of feed ingredients and the use of any mechanism to enhance availability of nutrients will benefit growth performance and reduce cost of production. The commercial exogenous enzymes used in the livestock feed industry are products of microbial fermentation. Feed enzymes are produced by a batch fermentation process, beginning with a seed culture and growth media. Once the fermentation is complete, the enzyme protein is separated from the fermentation residues and source organism. At weaning, piglets often suffer a growth check because of changes in their nutrition, environment and immune status. Sow's milk is replaced by a diet containing complex carbohydrates which necessitates a dramatic

change in endogenous enzyme secretion. A rapid development in the digestive tract has to take place and the combined effects of these changes can result in a temporary reduction in digestive competence, pre-disposing the young animal to mal-absorption, which may lead to scouring. The addition of exogenous enzymes can augment and complement those secreted endogenously to ensure a more complete digestion of feed (Okai and Boateng, 2007). Their inclusion has been shown to improve nutrient digestion and absorption and hence growth rate for a range of diets (Partridge and Hazzledine, 1997). Okai *et al.* (2002) fed "Optizyme" (a powdered feed enzyme) at 50 g/100 kg of feed to growing pigs and this proved effective in the digestion of a high fibre diet. In addition, enzymes, such as α-galactosidases, pentosanases and proteases, can specifically target anti-nutritional factors, which impair digestion and therefore provoke digestive disturbances.

2.2.4 GROWTH-PROMOTING MINERALS

Elemental copper is a required nutrient for normal pig growth and is routinely added to swine diets at the rate of 6 to 11 ppm to meet this requirement. Zinc is an essential trace mineral for animals and it has been found to have antibacterial properties. Zinc is required for the immune system, for reproduction and for regeneration of keratin. High doses of Cu (125 to 250 ppm) and Zn (2000 to 3000 ppm) when added to feed have antimicrobial activities.

Copper is usually supplied as copper sulphate for very young piglets and the improvement in production (growth and feed conversion) is similar to that obtained with antibiotics (Cromwell, 1991). In growing pigs the results are also positive, albeit of a lesser magnitude. The most used form of zinc is zinc oxide, and the results obtained on growth and feed conversion in piglets, especially during the first weeks

post-weaning, are encouraging. However, in both cases, the mechanisms of action have not been well studied. Katouli *et al*. (2000) have recently published research demonstrating that zinc oxide is beneficial to the piglet's microflora equilibrium and that this applies particularly to coliforms during the first two weeks of life. Copper sulphate, when fed in excess of 300 to 500 ppm for an extended period of time, may be toxic. The severity of the toxicity is directly related to the level fed and is increased if the diets are low in zinc and iron. Additionally, these high zinc levels might be toxic for the adult pig and, therefore, its use is restricted to diets supplied for not more than two weeks after weaning.

Copper and zinc pose a severe problem of environmental contamination. To solve this, organic sources of these minerals, favouring their absorption from the gut and a decrease of Cu and Zn in the faeces, have been suggested by some researchers. However, the higher cost of the organic substitutes and the lack of data supporting a potential growth promotion effect may make this option unlikely. The response to copper can be observed in both the presence and absence of antibiotics; therefore, it cannot truly be considered an alternative for replacing antibiotics.

2.2.5 SYNBIOTICS

Another way to modify pig microflora is the use of synbiotics, which is the use of probiotics and prebiotics in combination (Gibson and Robberfroid, 1995). The live bacteria must be used with specific substrates for growth. Therefore, the colonization by an exogenous probiotic could be enhanced and extended by simultaneous administration of a prebiotic being specifically used by the probiotic strain as a substrate in the intestinal tract (Rolfe, 2000). Although works with synbiotics in pigs

are still scarce, results are promising. A few studies have shown that feeding a diet with synbiotics to young pigs increased *Lactobacillus* and *Bifidobacterium* levels when compared to prebiotics and probiotics alone (Nemcová *et al*, 1999). The administration to weanling pigs of *Lactobacillus paracasei* in addition to oligofructose resulted in higher numbers of total anerobes, aerobes and *Lactobacilli*, with a decrease in enterobacteria and clostridia (Nemcova *et al*., 1999). Estrada *et al.* (2001), feeding early-weaned pigs with FOS and *Bifidobacterium congum*, found an improvement in feed efficiency.

It seems that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut. However, there is little information on synbiotics and its possible mechanisms in young pigs.

2.2.6 DIRECT-FED MICROBIALS (DFM) OR PROBIOTICS

A DFM or probiotic is defined as " a preparation or a product containing viable, defined micro-organisms in sufficient number, which alter the microflora (by implantation or colonization) in a compartment of the host, and by that exert beneficial health effects on the host" (Roselli *et al*, 2005). According to Todd (2001) probiotics are natural live organisms either of bacteria or fungal cultures used as feed additives in livestock feeding and in human diets. According to the FAO/WHO (2001), probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host. The U.S. Food and Drug Administration (1998) defines a direct-fed microbial (DFM) as "a source of live (viable), naturally-occurring organisms" and currently requires manufacturers to use this term instead of probiotics. Direct-fed microbial products are available in a variety of forms including powders, liquids, pastes, gels, boluses and capsules. They may be administered

through feed, top-dressed, given as a paste or mixed into the drinking water or milk replacer. Handling instructions vary from single-dose to continuous feeding. Usually, DFM contain desirable gastrointestinal microbial cultures and/ or ingredients that may enhance the growth of desirable gastrointestinal microbes. While under normal conditions pathogenic organisms in the gut cannot grow and compete with the normal bacterial flora, during stress there is an upset in the microbial balance. Direct-fed microbial may establish a desirable balance of gastrointestinal organisms and/or the substances that contribute toward the balance. The effectiveness of DFM depends on when they are used. The best response can be observed in piglets after weaning or dietary changes, periods of stress and after antibiotic therapy. The main advantage of DFM is that, it doesn't leave residues in animal products, in contrast to antibiotics which could have serious consequences such as drug resistance (Abe *et al*., 1995). Therefore, some researchers have routinely replaced antibiotics with probiotics as therapeutic and growth promoting agents (Martins *et al*., 2005).

(i) MICROOGANISMS USED IN DFM

The definition of DFM covers yeast/fungi and bacterial strains. Several strains of bacteria, fungi or yeast have been used efficiently to produce different types of DFM. Various microorganisms that could be used as probiotics were isolated from gastrointestinal content, mouth and faeces of animals. The major microorganisms presently used as probiotics strains for animals are *Lactobacillus, Bifidobacterium, Bacillus spp, Streptococcus* and *Saccharomyces cerevisiae*. Shown in Table 2 below is a list of micro-organisms approved by the FDA and AAFCO for use in DFM products. They should be non-pathogenic, gram-positive, acid resistant, strain

specific, anti-E. coli, bile resistant, viable/stable, and must adhere to the intestinal mucosa and contain a minimum of 30×10^9 colony forming unit per gram (Pal, 1999).

Table 2. Microorganisms that are approved by FDA and AAFCO 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*
Bacteroides amylophilus	*Lactobacillus cellobiosus*	*Saccharomyces cerevisiae*
Bacteroides capillosus	*Lactobacillus curvatus*	*Streptococcus cremoirs*
Bacteriodes ruminicola	*Lactobacillus delbrueckii*	*Streptococcus diacetilactis*
Bacteroides suis	*Lactobacillus fermentum*	*Streptococcus faecium*
Bifidobacterium adolescentis	*Lactobacillus helveticus*	*Streptococcus intermedius*
Bifidobacterium animalis	*Lactobacillus lactis*	*Streptococcus lactis*
Bifidobacterium bifidum	*Lactobacillus plantarum*	*Streptococcus thermophilus*

Source: Alliance Animal Health: Proven Performance from Innovative Nutrition®

Most of the works on probiotics in the literature involved using one (single) or two strains of beneficial bacteria. But multi-strains of bacteria may be more useful to proliferate lactic acid bacteria. The combinations of DFM strains could increase the beneficial health effects compared with individual strains, because of their synergistic adhesion effects (Collado and Sanz, 2007). Bonsu (2009) observed significantly ($P<0.05$) higher weight gains when he fed a DFM product containing *Lactobacillus*

sp, Bacillus sp and *Saccharomyces cerevisiae* to broiler chicks and recorded higher egg weight in layers. However, some experiments have failed to show consistent and beneficial responses. For instance, Okai (2008) used the same DFM product and Mazorite (a naturally-mined mineral product that contains a broad spectrum of metabolically active minerals and 74 trace minerals) and had no significant (P>0.05) effect on growth performance in the DFM-treated pigs.

In making health claims about the importance of DFM supplementation in diets, it was necessary to conduct an evaluation of the quality, safety and effectiveness of DFM. Guidelines for the evaluation of DFM in feed have been outlined by the FAO (2002) as follows:

i. A DFM must be alive when administered.
ii. A DFM must have undergone controlled evaluation to document health benefits in the target host.
iii. A DFM must be a taxonomically defined microbe or combination of microbes (genus, species and strain level).
iv. A DFM must be safe for its intended use.

Based on the several strains of bacteria, fungi or yeast used in the preparation of DFM, there are two main types of DFM.

(ii) BACTERIAL DFM

Basically, two groups are used: The lactic acid bacteria group mainly *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus cellobiosus, Lactobacillus reuteri, Propionibacterium freudenreichii, Propionibacterium shermanii,* etc and bacteria belonging to the genus, *Bacillus* (i.e. *Bacillus coagulans, Bacillus lentus, Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis,* etc). Among these bacteria,

Lactobacillus (lactic acid bacteria) is the commonest in probiotics. Probiotic supplementation of intestinal microflora in poultry, especially with *Lactobacillus* species, showed beneficial effects on resistance to infectious agents such as *Escherichia coli, Salmonella sp.,* and more recently, *Eimeria acervulina* (Dallou *et al*., 2003). Pollmann *et al*. (1990) confirmed an improvement in ADG (11.0%) and feed conversion (1.5%) when *Lactobacillus acidophilus* was included in the diet of 7 kg pigs. The genus *Bacillus*, is one type of probiotic commonly in use today (Hong *et al*., 2005). These probiotics are primarily used in their spore form and have demonstrated beneficial effects in the prevention of gastrointestinal disorders (Hong *et al*., 2005). Yet, interactions of *Bacillus spp*. in the gastrointestinal system are complex and not well understood. Several studies have been completed with pigs using the BioPlus® 2B supplement (containing *Bacillus licheniformis* and *Bacillus subtilis* spores) which were shown to contribute to improved sow and piglet performance (Alexopoulos *et al*., 2004). Several *Bacilli spp*. have also been suggested to serve as a probiotic in broiler chickens. Barbosa *et al*. (2005) isolated several *Bacilli spp.* from the chicken gut and all strains examined demonstrated the ability to sporulate efficiently in the laboratory setting, to tolerate simulated gastrointestinal conditions and to exhibit antimicrobial activity against a broad spectrum of bacteria, including: *Clostridium perfringens, Listeria monocytogenes, and Staphylococcus aureus.*

(iii) FUNGAL/ YEAST DFM

Probably the first microorganisms used as DFM feed additives for domestic livestock were yeasts. By far the most commonly used yeast in animal feeding is *Saccharomyces cerevisiae.* "*Saccharomyces*" is derived from Greek and means "sugar mold" and "*cerevisiae*" comes from Latin and means "of beer". It is a species of

budding yeast. It is perhaps the most useful yeast owing to its use since ancient times in baking and brewing. It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-coloured fruits such as plums and exists among the waxes of the cuticle). Mostly, yeast cells used as DFM are produced through simple fermentation and culture methods.

The yeast, *Saccharomyces cerevisiae*, has shown promising effects on increasing the digestibility of feeds and the fibre fractions of feeds thereby increasing the availability of nutrients for animal productivity (Maurya *et al*, 1993). Matthew *et al* (1998) reported that the supplementation of live yeast culture improves growth performance in weanling pigs. Fungal fermentation extracts from *Aspergillus oryzae* are also used as DFM feed additives. Addition of *Aspergillus oryzae* increases the digestibility of feed and in particular hemicelluloses. *Saccharomyces cerevisiae* and *Aspergillus oryzae* provide a source of exogenous enzymes (which aid digestion) and B-vitamins which aid ruminal fermentation and improve animal performance. Regardless of all these benefits, some researchers have found inconsistencies in the effects of the use of live yeast cultures as feed additives in livestock production. For instance, Kornegay *et al*, (1995) reported that the addition of live yeast culture to the feed of swine could not show beneficial effect on the digestibility of nutrients. However, feeding an antibiotic-free creep feed supplemented multi-probiotic strains (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bacillus subtilis*, *Saccharomyces cerevisiae*) to suckling piglets resulted in increased body weight gain (Shim, 2005).

(iv) DFM's MECHANISMS OF ACTION

It has been suggested that probiotics are strain-specific and dose-specific. There are several proposed mechanisms by which probiotics may protect the host from intestinal disorder (Rolfe, 2000). These are:

i. Production of organic acids: DFMs have been found to produce a number of organic acids. The most common are lactic, acetic, and formic acids, which inhibit intestinal pathogens. Organic acids also serve as energy sources to the animal or other beneficial bacteria.

ii. Production of antimicrobials: Research has reported that certain strains of bacteria produce bacteriocins, antibiotics, hydrogen peroxide, and other compounds that inhibit intestinal pathogens.

iii. Enzyme activity: Beneficial bacteria, especially *Bacillus*, produce a variety of enzymes. Proteases, amylases, lipases, and glycosidases are just a few of the enzymes which may be produced. This may also explain improvements in feed efficiency that has been observed when certain DFMs are fed. *Bifidobacterium bifidum* produces a DNA polymerase that has been reported to be important in repairing damaged cells.

iv. Competitive exclusion: Beneficial microorganisms inhibit growth of potentially pathogenic microorganisms by competitive exclusion. Competitive exclusion is the use of space and nutrients by one organism, thereby denying the use of these commodities to another organism. Competitive exclusion of commensal micro-flora against pathogens include lowering pH through production of lactate, lactic acid and volatile fatty acids (VFA), competing for gut lining attachment and

available nutrients, producing bacteriocins and stimulating the immune system through cell wall components. Others include increasing the production of VFA, which have bacteriostatic and bactericidal properties and stimulating intraepithelial lymphocytes, and natural killer cells (Ishizuka *et al*., 2004).

v. Stimulation of immune response: Research has reported that when animals are fed certain strains of bacteria, the activity of their immune systems increases (Choudhari *et al*., 2008).

vi. Reductions of toxic amines: Amines, produced by some intestinal microbes, are irritating and toxic, and have been associated with diarrhoea. Lactic acid bacteria have been found to reduce the level of amines in the gut and to neutralize enterotoxins.

(v) IMPORTANCE OF DFM

Micro-organisms begin to colonize the sterile gastro-intestinal tract of the newborn pig right after birth and a fully developed gut micro-flora is established within weeks. The normal gut micro-flora is a complex ecosystem, habituated by up to 500 different species, which has a balanced co-existence with the host (Van Kessel *et al*., 2004). The composition of microbial community has a major influence on the degradation of the feed and builds a natural barrier against undesired micro-organisms. The species in the gut micro-flora have stimulating and/or depressing effects on each other and interact with the host animal in different ways and with different magnitude depending on age, feed composition, stress level, the flow of digesta, pH, molecular oxygen and oxidation/reduction potential and other environmental factors (Stewart *et al*., 1993). Any change in composition or density of the gut micro-flora, either during

its development or when already matured, increases the likelihood of instability and can affect sub-optimal production results.

Probiotics with scientifically proven efficacy and documented stability can provide reliable solutions to maintain gastro-intestinal integrity and thereby improve pig production. The conventional use of probiotics to modulate gastrointestinal health, such as improving lactose intolerance, increasing natural resistance to infectious diseases in the gastrointestinal tract, suppressing diarrhoea and reducing bloating, has been well investigated and documented (Liong, 2007).

(vi) EFFECTS OF DFM ON THE GASTROINTESTINAL MICROFLORA

Almost immediately after birth, the gastrointestinal tract is populated by an exceptionally different commensal bacterial population. These bacteria allow the digestion of compounds, such as cellulose, that require specific sets of enzymes. The bacteria gain from the stable synergistic habitat and the energy provided by ingested food (Macpherson and Harris, 2004). The balance between beneficial and pathogenic bacteria, within this flora, is a characteristic of a normally functioning gastro-intestinal tract. In addition to the beneficial effect on access to nutrients, the bacteria seem to have an action on intestinal physiology, morphology, mucus secretion, metabolism and immune functions (Shirkey *et al*., 2006). Germ-free animals have an undeveloped mucosal immune system with hypoplastic Peyer's patches and a reduced number of IgA-producing plasma cells in the lamina propia (Macpherson and Harris, 2004). When the organism experiences a stressor, the balance is altered and intestinal disorders occur that impact nutrient conversion, average daily gain and survival rate. As a result, one of the main reasons for using probiotics is to stabilize the digestive microflora and for them to compete with pathogenic bacteria. Several reports

demonstrated that administering lactic acid bacteria has an influence on the gastrointestinal microflora (Pollmann *et al*., 1990; Newman *et al*., 1990). The stimulation of the growth of both *Bifidobacteria* and *Lactobacilli* by supplementing multi-strain probiotics may help to protect young pigs against potential pathogens. The exact mechanism by which commensal bacteria exert their positive effect is still unknown. One hypothesis is that commensal bacteria compete with pathogenic bacteria for adhesion to common receptors in the intestinal epithelium; this phenomenon as described earlier is termed competitive exclusion (Schierack *et al*., 2006).

(vii) EFFECTS OF DFM ON NUTRIENT SYNTHESIS AND DIGESTIBILITY

The intestine is an organ that must meet two seemingly incompatible goals i.e. to maximize nutrient uptake and to minimize antigenic insult while tolerating the presence of indigenous microbiota and other antigens introduced by the presence of feed within the intestinal tract. Both of these functions require a number of faceted interactions between numerous physiological systems such as beneficial microbiota, the physical GIT barrier and the GIT immune system. The synchronization of these systems is crucial to maintaining nutrient uptake and utilization. Direct-fed microbials enhance nutrient utilization, synthesis, digestibility and production performance characteristics. Thus, nutrient utilization is improved by reducing the competition between the host and its enteric pathogenic microflora (Santos *et al.,* 2005). Fermentation of feed with lactic acid bacteria has been shown to increase folic acid content. In addition to nutrient synthesis, probiotics may improve the digestibility of some dietary nutrients such as protein and fat (Friend and Shahani, 1984). However, other reports show no effect on digestibility of Dry Matter (DM), Neutral

Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and amino acid when pigs were fed probiotics containing *Lactobacillus* or *Bacillus* cultures (Kornegay *et al*., 1995).

(viii) GROWTH PERFORMANCE EFFECTS OF DFM

Swine growth rate is one of the key indicators affecting the profitability of pork production. Improvement in growth rate and feed to gain ratio will result in improved profitability due to greater output and reduction in overhead costs. It is well known that the age and weight at weaning are closely related to post weaning growth rates. Many studies have demonstrated that weaning weight influences post weaning growth performance and also influences performance during the subsequent grower and finisher phases. An increase in pig weight at weaning of 1 kg will result in a pig which reaches slaughter weight at least 10 days faster. It is also accepted that average daily gain during the first week post-weaning has a major impact on subsequent growth performance (Cole and Cole, 2001). Piglets showed improved weight gain and feed efficiency when fed *Lactobacillus*-based probiotics (Cho *et al*., 1992). Several studies have shown that dietary supplementation of *Lactobacillus* and *Bacillus sp*. cause an increase in the growth performance of nursery pigs (Abe *et al*., 1995; Collinder *et al*., 2000). However, these results have not been consistent with some other studies which showed no response in the growth performance of nursery pigs in response to supplementation with *Lactobacillus sp*. or with *Bacillus sp*. (Jonsson and Conway, 1992).

Probiotics are beneficial to the host animal by increasing competition for adhesion receptors and nutrients with the pathogenic bacteria in the gut besides producing antibacterial substances which help in controlling the pathogenic gut microflora (Fuller, 1989). However, some other factors can complicate the effects of probiotics;

these include the environmental conditions of the research site, handling of the animals, genetic background of the animals, different stress factors, composition of gut microflora in the animals and chances for cross-contamination (Jonsson and Conway, 1992). Types of microorganisms and carriers in probiotics can also cause modifications in gut microorganism populations and as a result intestinal health modifications.

(ix) THE EFFECTS OF DFM's ON THE IMMUNE SYSTEM

The gastrointestinal tract is one of the places most exposed to pathogenic micro-organisms and non-viable materials including antigens and carcinogens. Fortunately, the intestinal mucosa functions to serve as an active barrier in the defence against the continuous challenge of food antigens and pathogenic microorganisms which are continually entering the intestinal tract. Aside from immune system protection, harmful agents are also cleared from the gut by the actions of gastric acid, peristalsis, mucus, intestinal proteolysis and the intestinal biota (Nava *et al*., 2005).

However, some foreign micro-organisms (pathogenic) and cell fragments do penetrate the gut wall by translocation through the epithelial layer or through Peyer's patches. These pathogenic micro-organisms cause diseases and decrease in the immune response of the host.

There has been a hypothesis that beneficial bacterial communities in the gut lead to positive effects on host health and this has led to the development of therapeutics that are based on the consumption of beneficial bacterial cultures (Nava *et al*., 2005). *Lactobacilli* are able to cross the intestinal mucous layer and they can survive in the spleen or in other organs for many days where they stimulate enzyme and phagocytic activities. Subcutaneous inoculation of *Lactobacillus casei* stimulated the production

of specific antibodies against *Pseudomonas* antigens by increasing the circulating IgM antibodies. Oral application of *Lactobacilli* led to macrophage and lymphocyte stimulation and to the release of the enzymes from murine peritoneal macrophages (Perdigón *et al*., 1990).

The mechanisms by which probiotic bacteria could mediate changes in the GIT are not well understood. However, Nava *et al*. (2005) suggested that probiotics may mediate their action by competing for intestinal epithelium adhesion receptors and by the production of antibacterial substances (e.g., bacteriocins or colicins) which modulate immune responses.

(x) OTHER EFFECTS OF DFM

Some other benefits include: increased resistance to infectious diseases, particularly of the intestine, decreased duration of diarrhoea, reduction in blood pressure (Sawada *et al*., 1990), reduction in serum cholesterol concentration (Drouault *et al*., 2002), reduction in allergy, stimulation of phagocytosis by peripheral blood leucocytes, modulation of cytokine gene expression (Perdigón *et al*., 1990), adjuvant effects, regression of tumors and reduction in carcinogen or co-carcinogen production (Goldin, 1998).

2.3 SUMMARY OF LITERATURE REVIEWED

Based on the literature reviewed the following inferences can be made: swine diets supplemented with feed additives led to efficient and profitable performance under good management conditions. Feed additives stimulate growth by improving the efficiency of feed utilization and weight gains; and provide health benefits by reducing mortality and morbidity from clinical and subclinical infections of the pig.

Traditionally, the feed additive market has been dominated by antibiotic growth promoters. Swine performance is potentially improved by using sub-therapeutic concentrations of antibiotic to increase rate of gain and / or improve feed conversion efficiency. However, the recent ban of antibiotics for this purpose in some developed countries as a result of antibiotic residues in meat**,** vegetables, manure, soil, air and surface waters, and the development of drug-resistant bacteria have stimulated research efforts to identify alternatives to their use as feed additives.

Direct-fed microbials or probiotics, prebiotics (oligosaccharides) and synbiotics are natural feed additives that have received increasing attention. Oligosaccharides promote the proliferation of beneficial bacterial population in the gastrointestinal tract by acting as a source of energy. This helps beneficial microorganisms to overcome pathogens in the GIT of the animal. Direct-fed microbials contain either cultures of live bacteria or fungi or both. They contribute to the intestinal microbiota by introducing live microorganisms into the GIT. These microorganisms confer health benefits to their host and also produce certain acids and enzymes to aid digestion of fibre. The studies described above suggest that DFM can either have positive or no effect on growth performance, feed conversion efficiency and the health of swine.

There is a dearth of information on the effects of DFM on pigs in the tropics. One recent study showed that the addition of DFM and Mazorite to the diets of the pigs did not seem to improve the growth performance and feed efficiency significantly. The current study was therefore conducted to provide further evidence on the effects of a DFM preparation containing *Lactobacillus sp, Bacillus sp* and *Saccharomyces cerevisiae* on the growth performance, blood profile and carcass characteristics of pigs.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE AND DURATION

The study, which covered a period of twenty-one weeks, 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 maximum and minimum monthly mean temperatures of the area during the project were 32.34°C and 22.62°C respectively with a mean relative humidity of 83.8%.

3.2 EXPERIMENTAL ANIMALS AND DESIGN OF EXPERIMENT

Forty-eight (48) Large White starter pigs (24 barrows and 24 gilts) with an overall mean initial liveweight of 10.38 kg were selected and randomly allocated to three dietary treatments (designated as CONTROL, DFM-1, and DFM-2) based on their sex and liveweight. Each treatment had sixteen (16) pigs and was replicated four (4) times with each replicate consisting of two (2) barrows and two (2) gilts. The experimental design used was Randomized Complete Block Design. For easy identification, all the pigs were tattooed.

3.3 HOUSING

The pigs were housed in a cement-block building with concrete floors and the building had corrugated aluminium roofing sheets. Internally, the building had 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 walls. The pens had

in-built feed and water troughs (180 x 40 cm) moulded with concrete on opposite walls. For the purpose of this study, the feed troughs were partitioned with pieces of bamboo stem to ensure uniformity of access to feed by the four pigs in each pen.

3.4 FEEDS AND FEEDING REGIME

The study was conducted in two phases, i.e. the Starter and the Grower-Finisher phases. The Starter phase lasted five (5) weeks and the diets offered each contained 18% CP whereas during the Grower-Finisher phase, the diets offered contained 16% CP (Table 3) and its duration was from the 6th week to the 21st week of the experiment. There was no inclusion of DFM in the CONTROL diet while DFM-1 and DFM-2 dietary treatments were the same diet but contained 1.5 and 3.0 ml DFM/kg diet respectively. This rate of DFM inclusion was the same for the two phases of the study, and the two levels of DFM were mixed with the feed at feeding time. The composition of the DFM product as indicated by the manufacturer is shown in Table 4. All pigs had *ad libitum* access to both feed and water.

3.5 MANAGEMENT

The pens, feed and water troughs were thoroughly washed, scrubbed and disinfected a few days before the commencement of the experiment and a foot bath was provided at the entrance of the building. The floors of the pens and water troughs were washed every morning and the feed troughs were emptied each morning and fresh feed was supplied. All the pigs were tattooed and dewormed (using Levamisole[1]) prior to the

[1] Levamisole (100mg): a dewormer (intramuscular administration). Dosage: 1ml per 20 kg body weight. Manufactured by Special T Products Ltd. 1 Liverpool Road, Maghull, Liverpool.Uk.

start of the experiment. A Pig was taken off the experiment when it attained a weight of 70 ± 0.5 kg at the weekly weighing.

Table 3: Composition (%) of the Experimental Diets*

Ingredients, (%)	Starter	Grower-Finisher
Maize	60	60
Wheat bran	22	25
Soyabean meal	8	8
Fishmeal	9	6
Vit-min. premix[#]	0.25	0.25
Common salt	0.25	0.25
Dicalcium phosphate	0.25	0.25
Oyster shell	0.25	0.25
Total	**100**	**100**
Composition (calculated)		
Digestible energy (kcal/kg)	3,217	3,193
Crude protein (%)	18	16
Calcium (%)	0.76	0.72
Phosphorus (%)	0.57	0.46
Composition (analyzed)		
Dry matter (%)	90.21	90.31
Crude protein (%)	19.17	17.47
Crude fibre (%)	4.3	5.6
Ether extract (%)	0.9	0.5
Ash (%)	4.35	4.83
NFE (%)	61.49	61.91

*The diets shown above were the Control diets for the Starter and Grower-Finisher phases. The DFM-1 and DFM-2 dietary treatments were the same diet but contained 1.5 and 3.0 ml DFM/kg diet respectively. [#]Vit-min. Premix per 100kg diet: VitaminA ($8x10^5$U.I); VitaminD3 ($1.5x10^4$U.I); VitaminE (250mg); VitaminK (100mg); VitaminB2 ($2x10^2$mg); VitaminB12 (0.5mg); Folic acid (50mg); Nicotinic acid ($8x10^2$mg); Calcium panthotenate (200mg); Choline ($5x10^3$mg). Trace elements: Mg ($5x10^3$mg); Zn ($4x10^3$mg); Cu ($4.5x10^2$mg); Co (10mg); I (100mg); Se (10mg). Antioxidants: Butylated hydroxytoluene ($1x10^3$mg). Carrier: Calcium carbonate q.s.p (0.25kg).

3.6 PARAMETERS MEASURED

3.6.1 FEED INTAKE

The left-over feeds were weighed using Camry scale[2]. Daily feed intake for each pig was determined by deducting the left-over feed from the quantity offered the previous

[2] Camry 25kg x 50g scale. Made in China.

day and the amount obtained divided by number of pigs that ate feed that day. Weekly and total feed intakes were computed from the daily feed intakes.

Table 4: Composition of the DFM[#]

Ingredients	Amount
Water	99.9%
Microorganisms*	
Lactobacillus sp	$1x10^8$ CFU/g
Bacillus sp	$4x10^{12}$ CFU/g
Saccharomyces cerevisiae	$11x10^5$ CFU/g

[#]Source: Basic Environmental Systems & Technology (BEST), Inc., Alberta, Canada.
*Apart from the fermentation products of the above microorganisms, the product may also contain the fermentation products of *Bacillus pumilus*, and *Lactobacillus paracasei*. The following minerals may also be present: Calcium (<0.02%); Sodium (<0.02%); Potassium (<0.005%); Magnesium (<0.003%); Molybdenum (<0.3ppm); Copper (<0.3ppm); Iron (<3ppm); Boron (<3ppm); Zinc (<2ppm).

3.6.2 LIVE WEIGHT CHANGES AND WEIGHT GAINS

Individually, all pigs were weighed weekly (Friday mornings) in a 200kg x 500g Gascoigne Precision Scale[3] to obtain their weekly liveweight changes. The total weight gain of each pig was divided by the pig's total number of days on the experiment to attain the daily liveweight gain.

3.6.3 FEED CONVERSION EFFICIENCY (FCE)

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

3.6.4 FEED COST AND ECONOMY OF GAIN

Cost per kg of feed was computed for the three diets used in the Starter and Grower-Finisher phases. In addition, the costs per kg inclusion of DFM were added to obtain

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

the cost per kg of DFM-1 and DFM-2 diets. Feed cost per kg gain for each diet was computed by finding the product of the cost per kg feed and FCE.

3.6.5 CARCASS PARAMETERS

The carcass parameters determined were:

i. Dressed weight and dressing percentage: The dressed weight is the warm carcass weight measured after evisceration and removal of the head and trotters. The dressing percentage was then calculated by expressing the dressed weights as percentage of the liveweight at slaughter.

ii. Viscera: was the weight of the entire viscera together with its contents.

iii. Weight of respiratory tract: This was the composite weight of the lungs, trachea and larynx.

iv. Weight of full GIT: This was measured as the weight of the full length of the GIT together with its contents.

v. Weight of empty GIT: This was the weight of the full length of the GIT without it contents.

vi. Weight of empty stomach: This was obtained by measuring the weight of the stomach minus its contents.

vii. Weight of the liver, spleen, heart, kidneys, trotters and head. The weights of the above-mentioned organs were measured on the day of slaughter.

After chilling at 4^{o}C for 24 hours, the following parameters were determined:

viii. Chilled weight: This was the cold carcass weight. Each carcass was then split into two along the vertebral column and the following measurements were taken on the right side of each carcass: carcass length, mean backfat thickness,

P_2, and loin eye area. The weights of the leaf fat, fillet, belly, loin, shoulder and thigh were taken as well.

ix. The mean backfat thickness: This was determined as the average of the backfat thickness measured at the first rib, last rib and last lumbar vertebrae.

x. Carcass length: This was measured in a straight line from the forward edge of the first rib to the forward edge of the aitch bone.

xi. Loin eye area: This was calculated by tracing the area of the *Longissimus dorsi* muscle (sectioned between the 12^{th} and 13^{th} ribs) and measuring with a planimeter[4].

xii. P_2: This was obtained by measuring the backfat depth at the P_2 position which is taken 6.5 cm from the dorsal midline to the anterior portion of the last rib.

xiii. Carcass lean (%): percentage of carcass lean was estimated using equations proposed by Ray (2009), as indicated below.

Estimated carcass lean (Ibs) = 2+ [Carcass wt (Ibs) X 0.45] + [Loin eye area (in^2) X 5.0] – [Fat Depth, (in) X 11.0]

$$\text{Predicted \% carcass lean} = \frac{\text{Estimated carcass lean}}{\text{Warm Carcass Weight}} \times 100$$

3.7 CHEMICAL ANALYSIS

3.7.1 FEED SAMPLES

Analyses were carried out in the Nutrition Laboratory of the Department of Animal Science, KNUST., Kumasi. Starter and grower-finisher feed samples were collected weekly after feed compounding. The samples of feed were bulked for each phase (i.e. Starter and grower-finisher phases) and ground in a laboratory hammer mill (Christy and Norris Ltd., Chelmsford, UK) to pass through a 1 mm sieve before analyses. The

[4] Planimeter: An instrument manufactured by Albright, Britain. Manufacturers of survey instruments and equipment.

prepared samples were analysed for their proximate or chemical components (i.e. moisture, crude protein, ether extract, ash, and crude fibre) according to standard methods of the AOAC (1990). The calculation of nitrogen-free extract was made by adding the percentage values on dry matter basis of these analysed contents and subtracting the sum from 100%.

3.7.2 BLOOD SAMPLES

Blood samples were collected from thirty (30) out of the forty-eight (48) pigs used in the study and there were thus ten (10) pigs from each treatment. Selection was based on the early attainment of the final weight of 70±0.5 kg. Two samples were taken from each pig using heparinized vacutainer (Venoject, lithium heparin, Terumo Europe, Leuven, Belgium) and sterilized micro tubes. The first sample from each pig was subsequently analysed for haematological parameters whilst serum was obtained from the other sample for biochemical studies.

(i) HAEMATOLOGY

The haematology procedure used was the Complete Blood Count (**Tiezt,** 1995**).** Blood samples were filled into micro-capillary tubes, sealed and fixed in a slot of a Thermo-spectronic machine (Automated Analyzer). They were centrifuged at 3000 rpm for five (5) minutes. The following haematological parameters were determined: Haemoglobin (Hb), Haematocrit (HCT), Red Blood Cell (RBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Platelets, White Blood Cell (WBC), Mean Platelet Volume (MPV) and Procalcitonin (PCT).

(ii) BIOCHEMISTRY

Blood samples in the vacutainer tubes were allowed to clot by leaving them at room temperature for about 2-3 hours. They were centrifuged and spun at 3000 rpm for 5 minutes. The serum was pipetted into clean dried bottles, labelled accordingly and kept in a freezer at -20°C until the test was ready to be done. Prior to the test, the samples were allowed to thaw. Biochemical studies were performed using the colometric method (Tietz, 1995). This method makes use of samples, reagents, standard solutions and colorimeter capable of measuring absorbance at a specified wavelength. The biochemical parameters analyzed for were: total protein, albumin, cholesterol, magnesium, phosphorus, calcium, chloride, sodium, potassium and bilirubin.

3.8 STATISTICAL ANALYSIS

All data collected were subjected to the analysis of variance technique described in the Genstat Discovery Edition (2008) and differences between treatment means determined by the Least Significant Differences (LSD).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 HEALTH

Generally, all the experimental pigs enjoyed good health. However, there were few cases of diarrhoea resulting in inappetence. Three pigs from the Control treatment and one from the DFM-1 treatment had diarrhoea. Those on the Control diet were given Penstrep-injectable[5] because it became necessary to do so. The one on the DFM-1 treatment regained its good health without medication. It may be suggested that, the presence of *Lactobacillus* and *Bacillus* in the DFM controlled the incidence of diarrhoea. This is in agreement with the reports by Kyriakis *et al*. (1999), Zani *et al.* (1998) and Cho *et al*. (1992) who had supplemented piglets' diets with probiotics containing *Lactobacillus* and *Bacillus spp.* and observed that the incidence of diarrhoea decreased.

One of the pigs on the Control treatment was observed to be licking the floor often. When slaughtered, it was observed that the liver was larger than the normal size and was quite hard. The condition was described as cirrhosis of the liver by the resident veterinary technician. Cirrhosis is an irreversible liver damage characterized by scarring, or fibrosis, and widespread formation of nodules in the liver and according to Fischer (1974) and Gebely (2004); it can be caused by parasitic infections, nutrient deficiencies and toxins. No mortality was recorded during the entire experimental period.

[5]Penstrep-Injectable antibiotic: Benzylpenicillium procanium 200000IE/ml; dihydrostreptomycini sulfas-mg/ml.Dosage:0.5-2ml per 10kg of body weight for 3-5 days. Manufactured by Dopharma B.V., Zalmweg 24, 4941 VX Raamsdonksveer, The Netherlands.

4.2 GROWTH PERFORMANCE

4.2.1 STARTER PHASE

Table 5 shows the growth performance data obtained during the starter phase of the study while Figure 2 is a weekly graphical presentation of the feed intake during the starter phase.

(i) Feed intake

During the first and second weeks of the starter phase, feed intake by the pigs on the DFM-1 diet was slightly higher than for those on the Control diet. However, there was a change in trend from the third week to the end of the starter phase (Figure 2). Pigs on DFM-2 treatment had the least feed intake throughout the starter phase. The mean values for the total feed intake at the end of the starter phase were 31.08, 30.27 and 27.57 kg for dietary treatments Control, DFM-1 and DFM-2 respectively (Table 5).

Table 5. Growth performance of pigs on the three dietary treatments-Starter Phase.

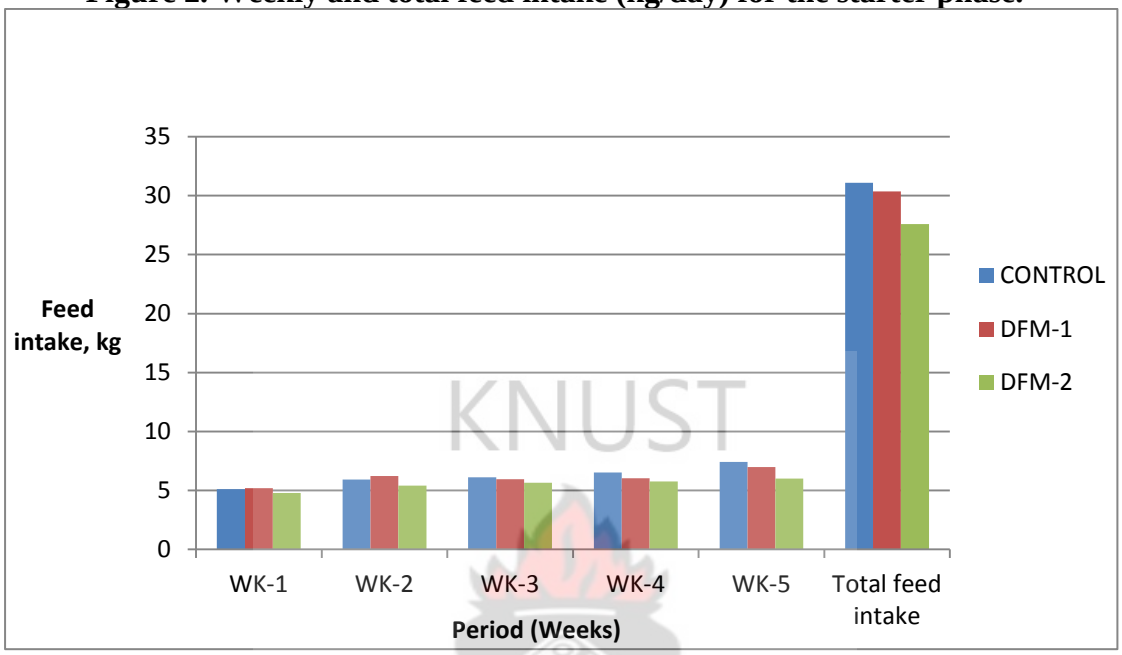
	TREATMENTS[ø]				
PARAMETER	CONTROL	DFM-1	DFM-2	L.S.D	S.D
No. of pigs	16	16	16	-	-
Initial weight, kg	10.38	10.38	10.38	0.160	0.065
Final weight, kg	25.03	24.97	23.03	2.478	1.013
Weight gain, kg	14.66	14.59	12.66	2.509	1.026
Daily weight gain, kg	0.42	0.42	0.36	0.072	0.029
Total feed intake, kg	31.08	30.27	27.57	5.325	2.176
Daily feed intake, kg	0.89	0.86	0.79	0.152	0.062
F.C.E (feed/gain)	2.12	2.08	2.17	0.099	0.040
Feed cost per kg,GH¢	0.40	0.42	0.44	-	-

[ø]Means in a row with no superscript are not significantly (P>0.05) different.

There were no significant differences (P>0.05) between the values for these three treatments, although pigs on the control diet had the highest intake. Brown (2009) observed a similar trend when he fed the same DFM product at different levels (0.5, 1.0 and 1.5 ml DFM/kg feed) to starter-grower pigs. Bonsu (2009) also found no

significant difference in feed intake when he fed the same DFM product to broiler and layer chickens.

Figure 2. Weekly and total feed intake (kg/day) for the starter phase.

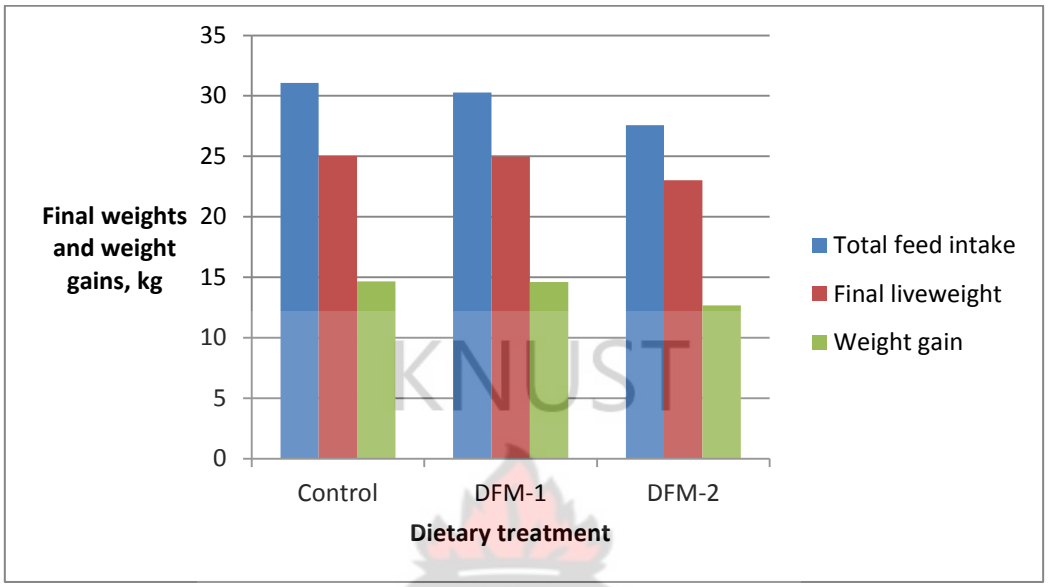


(ii) Liveweight changes (Initial, final liveweights and weight gains)

The pigs had mean initial liveweights of 10.38 kg across the three dietary treatments as shown in Table 5. At the end of the starter phase, the mean final weights recorded were 25.03, 24.97 and 23.03 kg for the Control, DFM-1 and DFM-2 treatments respectively. The weight gains recorded for the dietary treatments were therefore 14.66 (Control), 14.59 (DFM-1) and 12.66kg (DFM-2). There were no significant differences (P>0.05) in the final weights and weight gains. These results are in accord with the findings of Jost and Bracher (1999) and Brown (2009). They supplemented pigs' diets with commercial probiotics and did not observe any significant effect on weight gain. However, it is worth noting that the trend observed corresponds to that of the mean total and daily feed intake values (i.e. higher feed intake values corresponding to better weight gains) as shown in figure 3, which is a graphical

presentation of the mean total feed intake, final weight and weight gain for the starter phase.

Figure 3. Total feed intake, final weights and weight gains (kg)-starter phase.



(iii) Feed conversion efficiency and feed cost

The mean FCE values during the starter phase were not significantly (P>0.05) different for the three dietary treatments (Table 5). However, pigs on DFM-1 treatment had slightly better FCE than pigs on the Control and DFM-2 treatments. This result is in agreement with those found by Abe *et al*. (1995) and Kyriakis *et al*. (1999) who reported that, efficiency of gain of piglets is slightly improved by using DFM at 1.5%. Contrarily, Lantei (2008) found out that pigs on a Control diet (No DFM) were more efficient than those on DFM diets. Rao (2007) also had a similar result when he supplemented *Lactobacillus*-based DFM in the diet of nursery pigs. There were no significant differences (P>0.05) in FCE between the treatments.

The cost per kg of feed were GH¢ 0.40, GH¢ 0.42 and GH¢ 0.44 for the Control, DFM-1 and DFM-2 dietary treatments respectively. Feed cost for DFM diets included

the cost of inclusion of DFM which was GH¢14.67 per litre. Feed cost increased as the DFM level in the diets increased.

4.2.2 GROWER-FINISHER PHASE

The performance data during the grower-finisher phase of the study is presented in Table 6.

(i) Feed intake

The mean daily and total feed intakes were 2.02, 1.92 and 1.89 kg (DFI) and 157.70, 154.70 and 161.30 kg (TFI) for the Control, DFM-1 and DFM-2 diets respectively (Table 6). Again there were no significant differences among them and the pattern is similar to the feed intake trends observed at the starter phase.

Table 6. Growth performance of pigs on the three dietary treatments-Grower-Finisher Phase.

	TREATMENTS[ø]				
PARAMETER	CONTROL	DFM-1	DFM-2	L.S.D	S.D
No. of pigs	16	16	16	-	-
Initial weight, kg	25.03	24.97	23.03	7.510	3.320
Final weight, kg	71.72	71.28	72.03	0.875	0.387
Weight gain, kg	48.20	46.30	49.00	7.100	3.140
Daily weight gain, kg	0.62	0.57	0.57	0.080	0.036
Total feed intake, kg	157.70	154.70	161.30	20.26	8.960
Daily feed intake, kg	2.02	1.92	1.89	0.248	0.110
F.C.E (feed/gain)	3.28	3.35	3.30	0.275	0.121
Feed cost, GH¢/kg	0.34	0.36	0.38	-	-

[ø]Means in a row with no superscript are not significantly (P>0.05) different.

(ii) Liveweight changes and gains

The mean initial weights of the pigs during the grower-finisher phase were 25.03, 24.97 and 23.03 kg for the Control, DFM-1 and DFM-2 diets respectively while the corresponding final weights were 71.72, 71.28 and 72.03 kg (Table 6). There were no significant (P>0.05) differences among the values for the three dietary treatments. The

mean daily weight gains of pigs were also not significantly (P>0.05) different from each other. This time pigs on the DFM-2 diet had the highest weight gains and this is probably due to their higher feed intake.

(iii) Feed conversion efficiency

As shown in Table 6, no significant (P>0.05) differences were observed in the FCE. However, pigs on the Control diet were the most efficient among the three dietary treatments. This is contrary to the trend in the starter phase where the DFM-1 diet was the most efficiently utilized in terms of feed: gain ratio. This is in agreement with Zimmerman (1986) who found that average feed conversion efficiency response to feed additives in starter pigs is better than in grower pigs. This is because starter pigs are more susceptible to stress and sub-clinical diseases and consequently show a greater response to growth-promoting feed additives.

4.2.3 OVERALL PERFORMANCE: STARTER- FINISHER

(i) Feed intake

The mean values for overall daily feed intake were 1.66, 1.60 and 1.54 kg for dietary treatments Control, DFM-1 and DFM-2 respectively (Table 7). There were no significant differences (P>0.05) among the means. In a previous rat study, using the same DFM product, there were no significant differences (P>0.05) in the mean feed intake among the dietary treatments (Okai, 2008). Furthermore, other research had reported similar results for mean daily feed intake of nursery and growing pigs (Rao, 2007; Kornegay, 1990). The mean values for total feed intake on the other hand were 188 (Control), 185.10 (DFM-1) and 187.40 kg (DFM-2). Pigs on the Control diet recorded the highest total feed intake followed by DFM-2 and DFM-1 though there were again no significant (P>0.05) differences among them. The trends for daily feed

intake and the total feed intake were different for the three dietary treatments. For instance, total feed intake for the DFM-2 diet was higher than that of the DFM-1 although it had a lower daily feed intake. This is because pigs on DFM-2 spent more days on the experiment as a result of decreased daily feed intake.

Table 7. Overall growth performance of pigs on the three dietary treatments.

PARAMETER	TREATMENTS[ø]				
	CONTROL	DFM-1	DFM-2	L.S.D	S.D
No. of pigs	16	16	16	-	-
Initial weight, kg	10.38	10.38	10.38	0.160	0.065
Final weight, kg	71.72	71.28	72.03	1.057	0.387
Duration (days)	113.80	115.90	121.60	8.030	3.280
Weight gain, kg	61.34	60.91	61.66	1.110	0.454
Daily weight gain, kg	0.54	0.53	0.51	0.039	0.016
Total feed intake, kg	188.00	185.10	187.40	7.810	3.190
Daily feed intake, kg	1.66	1.60	1.54	0.123	0.050
F.C.E (feed/gain)	3.07	3.04	3.04	0.164	0.067
Feed cost/kg GH¢	0.37	0.39	0.42	-	-
Feed cost/kg gain, GH¢	1.14 [a]	1.19 [a]	1.28 [b]	0.0634	0.026

[ø]a,b- Means in a row with similar or no superscript are not significantly (P>0.05) different.

(ii) Liveweight changes and gains

The mean final weights recorded at the end of the experiment for the Control, DFM-1 and DFM-2 diets were 71.72, 71.28 and 72.03 kg respectively. These values were again not significantly (P>0.05) different from each other though there were numerical differences. Lantei (2008) reported that there were no significant (P>0.05) differences in final weights among dietary treatments when he supplemented similar levels of DFM to the diets of growing pigs. Also, there were no significant (P>0.05) differences in the liveweight changes among the three dietary treatments. Again, Jonsson and Conway (1992) had reported similar results for growth performance of

nursery pigs fed a *Bacillus*-based probiotic. Therefore, the results of this study corroborate what Lantei (2008) and Jonsson and Conway (1992) had earlier reported.

(iii) Feed conversion efficiency and duration of experiment

There were no significant differences (P>0.05) among the mean values of FCE for the three dietary treatments (Table 7). Nevertheless, the DFM diets were the more efficiently utilised in terms of feed: gain ratio than the Control diet. These results are consistent with those found by Jonsson and Conway (1992) and Ahmad (2006) who reported no differences in feed conversion efficiency of chicken fed diets with *Lactobacillus* cultures. In contrast, other researchers have demonstrated positive effects of probiotics supplementation in the diets of monogastrics. There were improvements in the feed conversion efficiency in the reports by Alexopoulos *et al*, (2004) and Collinder *et al*. (2000).

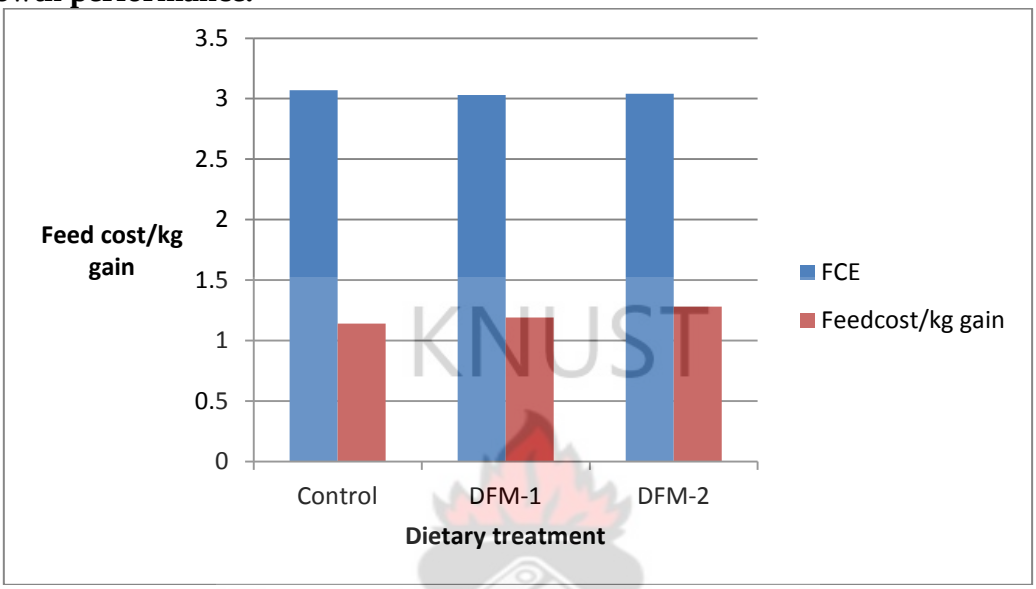
The duration of the experiment depended on when a particular pig in a treatment reached the targeted weight of 70±0.5 kg. The mean durations were 113.80, 115.90, and 121.60 days (Table 7) for the Control, DFM-1 and DFM-2 diets respectively. Although the differences between the means were not significant (P>0.05), pigs on Control and DFM-1 diets reached the market weight earlier than their counterparts on the DFM-2 diet.

(iv) Feed cost and economy of gain

The feed cost per kg of the Control, DFM-1 and DFM-2 diets were GH¢ 0.37, GH¢ 0.39 and GH¢ 0.42 respectively. The differences in the cost values were due to the inclusion of DFM in the diets and the feed cost (per kg) of diet increased with increasing level of dietary DFM addition. Pigs on the DFM-1 diets were more efficient with respect to feed to gain ratio (Table 7 and Fig. 4), however, it could be deduced that, it was more economical to raise pigs on the Control diet. Feed costs per

kg gain of the various diets were GH¢ 1.14 (Control), GH¢ 1.19 (DFM-1), and GH¢ 1.28 (DFM-2). There was a significant difference ($P<0.05$) between the value recorded for DFM-2 and the other dietary treatments (Table 7).

Figure 4. Feed conversion efficiency and Feed cost per kg gain for the overall growth performance.



4.3 CARCASS CHARACTERISTICS

Table 8 represents a summary of the carcass characteristics of the pigs fed the three different diets. The values shown are absolute values while relative values for some of these same parameters are shown in Table 9.

4.3.1 SLAUGHTER WEIGHT, DRESSED WEIGHT AND DRESSING PERCENTAGE

The mean liveweight of pigs on the three dietary treatments at the time of slaughter were 71.72, 71.28 and 72.03 kg for Control, DFM-1 and DFM-2 respectively. There were no significant ($P>0.05$) differences among these values. The mean warm dressed weights of 47, 46.94 and 47.31 kg for dietary treatments Control, DFM-1 and DFM-2 respectively did not elicit any significant ($P>0.05$) differences. A similar trend was

observed for the chilled dressed weights of 45.47 (Control), 45.47 (DFM-1) and 45.66 kg (DFM-2). Likewise, there were no significant (P>0.05) differences among the values for warm and chilled dressing percentages (Table 8).

Table 8: Mean carcass parameters of the pigs fed the three dietary treatments

PARAMETER	TREATMENTS[ø]				
	CONTROL	DFM-1	DFM-2	L.S.D	S.D
No. of pigs	16	16	16	-	-
Liveweight at slaughter, kg	71.72	71.28	72.03	1.057	0.432
Warm dressed weight, kg	47.00	46.94	47.31	1.259	0.515
Chilled dressed weight, kg	45.47	45.47	45.66	1.042	0.426
Warm dressing percentage	65.55	65.85	65.69	2.189	0.895
Chilled dressing percentage	63.41	63.79	63.39	1.925	0.787
Carcass length, cm	73.81	74.34	74.75	1.366	0.558
Loin eye area, cm^2	22.43	23.78	24.11	2.947	1.204
Fillet, kg	0.35	0.35	0.36	0.027	0.011
Leaf fat, kg	0.86	0.76	0.78	0.132	0.054
Respiratory tract, kg	1.21	1.18	1.20	0.076	0.031
Head, kg	4.93	4.82	4.84	0.302	0.123
Trotters[≠], kg	1.01	1.02	1.02	0.058	0.024
Viscera, kg	11.19	10.97	10.78	0.791	0.323
Full G.I.T., kg	7.84	7.67	7.59	0.658	0.269
Empty G.I.T., kg	2.99	2.79	2.89	0.209	0.086
Empty stomach, kg	0.52	0.50	0.49	0.084	0.035
Heart, kg	0.25	0.24	0.25	0.015	0.006
Kidney[≠], kg	0.24	0.24	0.24	0.027	0.011
Spleen, kg	0.15	0.16	0.14	0.047	0.019
Liver, kg	1.55	1.39	1.38	0.207	0.085
Belly, kg	4.52	4.53	4.47	0.386	0.158
Thigh, kg	6.79	6.67	6.79	0.191	0.078
Shoulder, kg	4.10	4.12	4.12	0.249	0.102
Loin, kg	6.19	6.17	6.08	0.375	0.153
Backfat thickness, cm	2.87	2.42	2.41	0.206	0.084
P_2 measurement, cm	2.29	2.02	1.98	0.151	0.062
Lean, kg	28.30	29.11	29.32	1.411	0.576
Predicted % carcass lean	60.79	62.43	61.72	3.121	1.275

[ø]Means in a row with no superscript are not significantly (P>0.05) different.
[≠]The mean weight for the trotters and the kidney are the mean values for the 4 trotters and 2 kidneys for each pig.

Apgar *et al* (1993) had earlier observed no significant differences (P>0.05) in dressed weight and dressing percentage of pigs when Probios (a commercial probiotic) containing *Bifidibacterium globosum A* (BGA) was supplemented in the diets of grower-finisher pigs. Similarly, Lantei (2008) had no significant differences (P>0.05) in dressed weight and dressing percentage when he supplemented the same DFM used in this study in the diets of starter-grower pigs.

4.3.2 ABSOLUTE AND RELATIVE WEIGHTS OF HEAD AND TROTTERS

The mean absolute head weights were 4.93, 4.82 and 4.84 kg for dietary treatments Control, DFM-1 and DFM-2 respectively. The Control had the highest mean head weight with those on the DFM-1 diets having the least. On the contrary, pigs on the DFM dietary treatments had slightly higher mean absolute trotter weights than their Control counterparts. These differences were however not statistically significant (P>0.05). There were also no significant (P>0.05) differences in their corresponding relative head and trotter weights (Table 9). This agrees with the findings of Saka (1984) who reported that these components do not differ much in their development except under severe conditions of malnutrition.

4.3.3 CARCASS LENGTH, BACKFAT THICKNESS, P_2 MEASUREMENT AND LOIN EYE AREA (LEA).

The mean carcass length, backfat thickness (BFT), P_2 measurement and loin eye area for the pigs on dietary treatments, Control, DFM-1 and DFM-2 were 73.81, 74.34 and 74.75 cm (carcass length); 2.87, 2.42 and 2.41 cm (BFT); 2.29, 2.02 and 1.98 cm (P_2) and 22.43, 23.78 and 24.11cm^2 (LEA) respectively (Table 8). The higher carcass length of pigs on the DFM diets observed here, corroborates the findings of Anna *et*

al., (2005) who observed significantly (P<0.05) higher carcass length in probiotic-supplemented group than in the non-supplemented group although in this instance, the differences were not significant (P<0.05).

Although there were no significant (P>0.05) differences among the mean values for backfat thickness, P_2 and loin eye area across the three dietary treatments, pigs on the DFM dietary treatments had larger loin areas and less fat (backfat thickness and P_2) in their carcasses.

4.3.4 LEAN AND PREDICTED PERCENTAGE CARCASS LEAN

The mean lean and predicted percentage carcass lean values for the three dietary treatments i.e. Control, DFM-1 and DFM-2 were 28.30, 29.11 and 29.32 kg (lean) and 60.79, 62.43 and 61.72 % (percentage carcass lean) respectively (Table 8). Although there were no significant (P>0.05) differences among the mean values for lean and predicted percentage carcass lean across the three dietary treatments, pigs on the DFM dietary treatments tended to have leaner carcasses because they had less backfat and P_2 measurements and larger loin eye areas. Adams *et al*. (1972) had earlier made a similar observations.

4.3.5 ABSOLUTE AND RELATIVE WEIGHT OF SHOULDER, THIGH, LOIN AND BELLY

The mean absolute weights of shoulder, thigh, loin and belly for the three dietary treatments i.e. Control, DFM-1 and DFM-2 were 4.10, 4.12 and 4.12 kg (shoulder); 6.79, 6.67 and 6.79 kg (thigh); 6.19, 6.17 and 6.08 kg (loin); and 4.52, 4.53 and 4.47 kg (belly) respectively (Table 8). There were no significant (P>0.05) differences among these values but as the data clearly indicates pigs on the Control diet had

slightly heavier loin and lower shoulder weights compared to their counterparts on the DFM diets. These results correspond with the findings of Lantei (2008) who stated that, pigs on a Control (No DFM) diet, compared to those on DFM diets, recorded higher and lower loin and shoulder weights respectively. The corresponding relative weights of shoulder, thigh, loin and belly as shown on Table 9 did not elicit any significant (P>0.05) differences .

Table 9: Mean relative (%) weights of some body components

PARAMETER	TREATMENTS[ø]				
	CONTROL	DFM-1	DFM-2	L.S.D	S.D
No. of pigs	16	16	16	-	-
Head	6.88	6.75	6.73	0.388	0.158
Trotters	1.41	1.43	1.41	0.081	0.033
Viscera	15.60	15.39	14.97	1.089	0.445
Fillet	0.48	0.50	0.49	0.040	0.016
Leaf fat	1.19	1.07	1.09	0.187	0.077
Respiratory tract	1.69	1.66	1.67	0.107	0.044
Full GIT	10.93	10.77	10.54	0.918	0.375
Empty GIT	4.16	3.91	4.02	0.300	0.123
Stomach (empty)	0.72	0.70	0.68	0.119	0.049
Heart	0.35	0.34	0.3	0.018	0.007
Kidney	0.34	0.33	0.33	0.038	0.015
Spleen	0.21	0.23	0.20	0.065	0.026
Liver	2.17	1.95	1.92	0.277	0.113
Belly	6.30	6.35	6.22	0.568	0.232
Thigh	9.47	9.36	9.43	0.361	0.147
Shoulder	5.71	5.79[a]	5.71	0.387	0.158
Loin	8.64	8.65	8.44	0.461	0.188

[ø]Means in a row with no superscript are not significantly (P>0.05) different.

4.3.6 ABSOLUTE AND RELATIVE WEIGHTS OF VISCERA, FULL GIT AND EMPTY GIT AND RESPIRATORY TRACT

The three dietary treatment effects on absolute viscera, full and empty GIT and respiratory tract weights were not significant (P>0.05). However, pigs on the Control diet had the highest viscera weight, as earlier observed by Lantei (2008) and Brown

(2009). The respiratory tract, full and empty GIT also followed the same trend with the Control group having the highest weights. Their corresponding relative weights did not deviate from the same trend as there were no significant differences between (P>0.05) them either.

4.3.7 ABSOLUTE AND RELATIVE WEIGHT OF LIVER, KIDNEY, SPLEEN, HEART AND EMPTY STOMACH

The mean absolute weights of liver, kidney, spleen, heart and empty stomach were 1.55, 1.39 and 1.38 kg (liver); 0.24, 0.24 and 0.24 kg (kidney); 0.15, 0.16 and 0.14 kg (spleen); 0.25, 0.24 and 0.25 kg (heart) and 0.52, 0.50 and 0.49 kg (empty stomach) for pigs fed the Control, DFM-1 and DFM-2 respectively (Table 8). There were no significant (P>0.05) differences among the means for the above parameters. The corresponding mean relative weights of liver, kidney, spleen, heart and empty stomach followed a similar trend (Table 9). Islam *et al.* (2004) supplemented probiotics (Protexin® Boost) in the diets of broiler chickens and did not record any significant differences in the weights of the internal organs.

4.3.8 ABSOLUTE AND RELATIVE WEIGHT OF FILLET AND LEAF FAT

The mean absolute weights of the fillet were 0.35 (Control), 0.35 (DFM-1) and 0.36 kg (DFM-2) and its corresponding mean relative weights were 0.48 (Control), 0.50 (DFM-1) and 0.49% (DFM-2). These values did not differ significantly (P>0.05) from each other. The mean absolute and relative weights of leaf fat were 0.86 kg, 1.19%; 0.76 kg, 1.07% and 0.78 kg, 1.09% for Control, DFM-1 and DFM-2 dietary treatments respectively. Pigs on the Control diet had more leaf fat compared to pigs on the DFM diets and this corroborates the findings of Apgar *et al* (1993), where

more backfat thickness and leaf fat were obtained in the control pigs. Adams *et al.* (1972) found that, pigs with smaller loin eye area are usually fatty.

4.4 BLOOD PROFILE

Information on the blood profile of pigs may be useful tools in assisting in their nutrition, health and management. As stated earlier, based on the early attainment of the final weight of 70±0.5 kg, 30 out of the 48 pigs were selected and used in the blood studies. The data obtained from the analyses of the blood for haematological and biochemical values are shown in Tables 10 and 11, respectively.

4.4.1 HAEMATOLOGICAL PARAMETERS

(i) HAEMOGLOBIN, HAEMATOCRIT AND RBC

The mean haemoglobin, haematocrit and RBC values were 12.58, 12.32 and 12.36 g/dl (haemoglobin); 35.91, 36.12 and 36.31 % (haematocrit) and 6.40, 6.18 and 6.35 (RBC, $x10^{12}$/l) for the dietary treatments, Control, DFM-1 and DFM-2 respectively (Table 10). There were no significant ($P > 0.05$) differences among the values across the three dietary treatments. This is in agreement with the findings of Rao (2007). He studied the effects of dietary supplementation of *Lactobacillus*-based probiotics on growth and gut environment of nursery pigs and observed no differences ($P > 0.05$) in red blood cell count, haematocrit, and haemoglobin among treatment groups. Also, the ranges of these values are in accord with the findings of Okai *et al.* (1995). They studied the haematological and serum biochemical patterns in Large White pigs raised in Ghana.

(ii) THE MCV, MCH, MCHC and MPV VALUES

As shown in Table 10, there were no significant (P > 0.05) differences among the dietary treatments in relation to mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and mean platelet volume. Moreover, all the values were within the reference range (Appendix Table vii) and this signifies that DFM supplementation did not have any significant effect on these parameters.

(iii) WBC AND PLATELETS

The mean WBC counts of the pigs were 14.32, 14.77 and 15.06 (x 10^9/L) for the Control, DFM-1 and DFM-2 diets respectively. The WBC counts were numerically higher in the DFM treatment groups compared to the Control (Table 10) but they were all within the normal reference range (Appendix Table vii). This is in agreement with the findings of Choudhari *et al*. (2008) who observed that, oral inoculation of young pigs with *Lactobacilli spp*. elevated serum proteins and white blood cell counts.

Table 10: Haematological values of pigs at slaughter

PARAMETER	TREATMENTS[ø]				
	CONTROL	DFM-1	DFM-2	L.S.D	S.D
No. of pigs	10	10	10	-	-
Haemoglobin, g/dl	12.58	12.32	12.36	1.126	0.536
Haematocrit, %	35.91	36.12	36.31	3.428	1.632
RBC, x10^{12}/l	6.40	6.18	6.35	0.644	0.306
MCV, fl	57.79	58.55	54.85	3.712	1.529
MCH, pg	19.60	19.90	23.90	8.110	3.86
MCHC, g/dl	34.00	34.03	32.89	2.324	1.106
Platelets, x10^9/l	206.00	180.00	242.00	83.600	39.8
WBC, x10^9/l	14.32	14.77	15.06	3.729	1.775
MPV, fl	8.80	8.57	8.78	0.769	0.366
PCT, %	0.18	0.15	0.22	0.076	0.036

[ø]Means in a row with no superscript are not significantly (P>0.05) different.

The capacity of probiotics to stimulate the immune system is one of its main advantages. Perdigon *et al*. (1990) suggested that *Lactobacillus casei* has

immunoadjuvant activity. The mean platelets values for the three dietary treatments were 206 (Control), 180 (DFM-1) and 242 fl (DFM-2) as shown in Table 10. However, statistically there were no significant (P>0.05) differences among the three dietary treatments with respect to the WBC and platelets counts.

4.4.2 SERUM BIOCHEMICAL PARAMETERS

(i) TOTAL PROTEIN AND ALBUMIN

No significant (P>0.05) differences were observed among the mean total protein values of 69.70, 68.50 and 74.10 g/l for the Control, DFM-1 and DFM-2 dietary treatments respectively (Table 11). On the other hand, there were significant (P<0.05) differences in the treatment means for DFM-1 and DFM-2 pigs in relation to albumin. DFM-1 pigs had the highest albumin value of 42.06 g/l followed by Control (40.03 g/l) and DFM-2 (38.42 g/l). The DFM-2 albumin value was within the reference range, whilst those of the Control and DFM-1 treatment were not (Appendix Table viii).

Table 11: Blood biochemical values of pigs at slaughter

PARAMETER	TREATMENTS[ø]				
	CONTROL	DFM-1	DFM-2	L.S.D	S.D
Total protein, g/l	69.70	68.50	74.10	15.140	7.491
Albumin, g/l	40.03[ab]	42.06 [a]	38.42[b]	3.362	1.632
Cholesterol, mmol/l	2.27	2.16	2.09	0.683	0.341
Magnesium, mmol/l	0.60	0.56	0.62	0.144	0.071
Phosphorus, mmol/l	1.75	1.99	1.96	0.484	0.239
Calcium, mmol/l	1.54	1.62	1.74	0.679	0.337
Chloride, mmol/l	75.00	83.40	82.00	20.620	10.26
Sodium, mmol/l	110.90	114.00	124.00	29.190	13.98
Potassium, mmol/l	4.37	4.39	4.80	0.794	0.412
Bilirubin, mmol/l	0.17	0.16	0.24	0.301	0.149

[ø]a,b- Means in a row with similar or no superscript are not significantly (P>0.05) different.

(ii) MAGNESIUM, PHOSPHORUS, CALCIUM, CHLORIDE, SODIUM, POTASSIUM AND BILIRUBIN

There were no significant (P>0.05) differences in the values obtained for the three dietary treatments for magnesium, phosphorus, calcium, chloride, sodium, potassium and bilirubin (Table 11). It has been hypothesized that *Lactobacilli* DFM may help correct malabsorption of trace minerals, particularly those diets high in phytate content from whole grains, nuts, and legumes (Famularo *et al*., 2005). Conversely, Shareef and Al-Dabbagh (2009) added probiotics at different levels in the diets of broiler chicks and found no effect on serum calcium and uric acid levels when compared with a control treatment.

(iii) CHOLESTEROL

Dietary supplementation of probiotics such as of *Lactobacillus spp*. has been shown to cause reduction in serum cholesterol concentration due to cholesterol assimilation by the *Lactobacilli* cells (Drouault *et al*, 2002). Commonly used probiotics also have been shown to have beneficial effects on cholesterol metabolism (Richardson *et al* 2003). As shown on Table 11, pigs on the DFM diets had lower mean cholesterol values than their Control counterparts although there were no significant differences (P>0.05) in the values observed.

CHAPTER FIVE

5.0 GENERAL SUMMARY

A summary of the results from this experiment shows that at the Starter phase, pigs on DFM-2 treatment had the lowest feed intake compared to their counterparts on the other treatments (i.e. Control and DFM-1). The mean daily feed intake at the end of the starter phase was 0.89, 0.86 and 0.79 kg for the Control, DFM-1 and DFM-2 dietary treatments respectively. This showed a trend of decreasing feed intake ($P>0.05$) with increasing levels of the DFM. This observation is consistent with results of Brown (2009).

There were no significant ($P>0.05$) effect of the DFM on the final weights and weight gains for the Starter phase. The mean daily weight gain at the end of the Starter phase was 0.42, 0.42 and 0.36 kg for the Control, DFM-1 and DFM-2 dietary treatments respectively. These results are in agreement with the findings of Jost and Bracher (1999) and Brown (2009) who stated that supplementation of pig diets with commercial probiotics did not result in any significant effect on weight gain. Efficiency of feed utilization during the Starter phase was not significantly ($P>0.05$) different for the three dietary treatments. However, pigs on DFM-1 treatment had slightly better FCE than pigs on the Control and DFM-2 treatments. This was as a result of a lower feed intake and a higher body weight gain. Abe *et al*. (1995) and Kyriakis *et al*. (1999) also reported of marginal improvement in efficiency of feed utilization when piglets were fed a DFM-incorporated diet. The costs per kg of feed were GH¢ 0.40, GH¢ 0.42 and GH¢ 0.44 for the Control, DFM-1 and DFM-2 dietary treatments respectively. Feed cost for DFM diets included the cost of inclusion of DFM which was GH¢14.67 per litre. Feed cost increased as the DFM level in the

diets increased. These translated into increasing feed cost per live weight gain as the level of DFM increased.

During the Grower-finisher phase, the mean daily and total feed intakes were 2.02, 1.92 and 1.89 kg (DFI) and 157.70, 154.70 and 161.30 kg (TFI) for the Control, DFM-1 and DFM-2 diets respectively. Again there were no significant differences among them. Also, this is similar to the feed intake trend at the Starter phase. The Control treatment had the highest intake followed by DFM-1 and then DFM-2. The mean final weights were 71.72 (Control), 71.28 (DFM-1) and 72.03 kg (DFM-2). There were no significant ($P>0.05$) differences among the three dietary treatments. The mean daily weight gains of pigs were also not significantly ($P>0.05$) different from each other. However, pigs on the DFM-2 diet had the highest weight gains. There were no significant ($P>0.05$) differences observed in the mean FCE values although pigs on the Control diet were the most efficient among the three dietary treatments. This is contrary to the trend in the Starter phase where DFM-1 diet was the most efficiently utilized in terms of feed: gain ratio. This is in agreement with Zimmerman (1986) who found that average feed conversion efficiency response to feed additives in starter pigs is better than in grower pigs.

The mean values of daily feed intake at the end of the study (Overall performance) also showed a trend of decreasing feed intake ($P>0.05$) with increasing levels of the DFM. Also there were no significant ($P>0.05$) differences in the mean daily weight changes and FCE for the three dietary treatments (Table 7). Feed cost per kg gain of the various diets were GH¢ 1.14 (Control), GH¢ 1.19 (DFM-1) and GH¢ 1.28 (DFM-2). The feed cost per kg gain increased ($P<0.05$) with increasing level of DFM. It could be deduced that, it was more economical to raise pigs on the Control diet.

There were no significant (P>0.05) differences amongst the various carcass parameters studied in this experiment. However, it is important to mention that loin eye area, backfat thickness, P_2 fat, and lean measurements followed a characteristic pattern with the addition of the DFM. Whilst the loin eye area and lean increased with increasing levels of the DFM, backfat thickness and P_2 measurement declined with increasing levels of the DFM. This suggests that the addition of DFM to the diets tended to promote lean tissue growth.

The mean haemoglobin, haematocrit and RBC values were not significant (P>0.05) among the three dietary treatments. Cholesterols levels were low among pigs on the DFM-dietary treatments. The DFM probably stimulated the immune systems (P>0.05) of the pigs on the DFM-diets by elevating their white blood cell counts. There was a significant (P<0.05) difference in the treatment means for DFM-1 and DFM-2 pigs in relation to albumin. DFM-1 pigs had the highest albumin value of 42.06 g/l followed by Control (40.03 g/l) and DFM-2 (38.42 g/l).

There have been inconsistency of results of feeding biological additives and this may be attributed to several factors such as; environmental conditions of the research site, handling of the animals, genetic background of the animals, different stress factors, composition of gut microflora in the animals and chances for cross-contamination (Jonsson and Conway, 1992). According to Prescott and Baggot (1993), growth promoters have been shown to perform best under unhygienic and poor living conditions like overcrowding. This study was carried out under reasonably good hygienic condition and this may ultimately account for the results obtained which suggest that DFM supplementation had no beneficial economic effects.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

This study investigated the potential effects of RE-3, a DFM product on the growth performance, blood profile and carcass characteristics of pigs.

At the starter phase, pigs on DFM-1 (1.5 ml DFM/kg diet) diet had slightly better FCE than their counterparts on the Control (no DFM) and DFM-2 (3 ml DFM/kg diet) diets, although the mean FCE values were not significantly ($P>0.05$) different for the three dietary treatments. Contrary to the trend in the starter phase where DFM-1 diet was the most efficiently utilized in terms of feed: gain ratios, pigs on the Control diet were the most efficient at the grower-finisher stage. The DFM did not have significant ($P>0.05$) effect on the various blood parameters although it slightly stimulated the immune system and there was a reduction in the mean cholesterol values.

Overall, it is more economical to raise pigs on the Control diet because it had the lowest feed cost per kg gain value. However, pigs on the DFM dietary treatments tended to have leaner carcasses than their counterparts on the Control diet.

Generally, it can be concluded that the supplementation of DFM in the diets of starter and grower-finisher pigs did not seem to improve growth performance, blood profile and carcass characteristics.

It is recommended that on-farm feeding trials be conducted to evaluate the effects of DFM supplementation of the diet of pigs under on-farm conditions.

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8.0 APPENDICES

Table (i). ANALYSIS OF VARIANCE (ANOVA) TABLES FOR SOME GROWTH PERFORMANCE TRAITS (STARTER PHASE)

a. INITIAL WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	45.608756	15.202919	1779.42		0.1599
TRT	2	0.000004	0.000002	0.00	1.000	
Residual	6	0.051263	0.008544			
Total	11	45.660023				

b. DAILY FEED INTAKE, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	2.6756	0.8919	7.21		0.152
TRT	2	0.3533	0.1767	1.43	0.311	
Residual	6	0.7423	0.1237			
Total	11	3.7713				

c. TOTAL FEED INTAKE, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	3277.6	1092.5	7.21		5.325
TRT	2	432.8	216.4	1.43	0.311	
Residual	6	909.3	151.6			
Total	11	4619.8				

d. DAILY GAIN, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	0.041152	0.013717	7.99		0.0717
TRT	2	0.008438	0.004219	2.46	0.166	
Residual	6	0.010303	0.001717			
Total	11	0.059893				

e. TOTAL WEIGHT GAIN, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	50.411	16.804	7.99		2.509
TRT	2	10.337	5.168	2.46	0.166	
Residual	6	12.621	2.103			
Total	11	73.369				

f. FCE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	0.66721	0.22240	4.26		0.0989
TRT	2	0.30268	0.15134	2.90	0.132	
Residual	6	0.31354	0.05226			
Total	11	1.28343				

g. FINAL WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
REPS stratum	3	186.233	62.078	30.28		2.478
TRT	2	10.344	5.172	2.52	0.160	
Residual	6	12.302	2.050			
Total	11	208.879				

Table (ii). ANALYSIS OF VARIANCE (ANOVA) TABLES FOR SOME GROWTH PERFORMANCE TRAITS (GROWER-FINISHER PHASE)

a. INITIAL WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	10.34	5.17	0.23	0.796	7.51
Residual	9	198.54	22.06			
Total	11	208.88				

b. DAILY FEED INTAKE, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	0.03609	0.01805	0.75	0.500	0.2483
Residual	9	0.21685	0.02409			
Total	11	0.25294				

c. TOTAL FEED INTAKE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	86.9	43.4	0.27	0.769	20.26
Residual	9	1444.0	160.4			
Total	11	1530.9				

d. DAILY WEIGHT GAIN, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	0.006097	0.003049	1.21	0.343	0.0804
Residual	9	0.022746	0.002527			
Total	11	0.028843				

e. TOTAL WEIGHT GAIN,kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	15.20	7.60	0.39	0.690	7.10
Residual	9	177.12	19.68			
Total	11	192.32				

f. FEED CONVERSION EFFICIENCY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	0.01255	0.00628	0.21	0.812	0.2745
Residual	9	0.26505	0.02945			
Total	11	0.27761				

g. FINAL WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Treat	2	1.1354	0.5677	1.90	0.205	0.875
Residual	9	2.6914	0.2990			
Total	11	3.8268				

Table (iii). ANALYSIS OF VARIANCE (ANOVA) TABLES FOR SOME GROWTH PERFORMANCE TRAITS (OVERALL PERFORMANCE)

a. DAILY FEED INTAKE, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.00159332	0.00053111	6.63		0.123
Trt	2	0.00052905	0.00026453	3.30	0.108	
Residual	6	0.00048061	0.00008010			
Total	11	0.00260298				

b. TOTAL FEED INTAKE, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	10.9718	3.6573	23.48		7.810
Trt	2	0.6418	0.3209	2.06	0.208	
Residual	6	0.9348	0.1558			
Total	11	12.5483				

c. DAILY WEIGHT GAIN, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0101979	0.0033993	6.58		0.03934
Trt	2	0.0025703	0.0012852	2.49	0.163	
Residual	6	0.0031013	0.0005169			
Total	11	0.0158695				

d. TOTAL WEIGHT GAIN, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	43.7227	14.5742	35.42		1.110
Trt	2	1.1354	0.5677	1.38	0.321	
Residual	6	2.4688	0.4115			
Total	11	47.3268				

e. DAYS TO SLAUGHTER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	1168.60	389.53	18.10		8.03
Trt	2	132.20	66.10	3.07	0.121	
Residual	6	129.14	21.52			
Total	11	1429.93				

f. FINAL WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.4518	0.1506	0.40		1.057
Trt	2	1.1354	0.5677	1.52	0.292	
Residual	6	2.2396	0.3733			
Total	11	3.8268				

g. FCE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.00033703	0.00011234	1.32		0.01594
Trt	2	0.00007629	0.00003815	0.45	0.658	
Residual	6	0.00050908	0.00008485			
Total	11	0.00092240				

Table (iv). ANALYSIS OF VARIANCE (ANOVA) TABLES FOR SOME CARCASS CHARACTERISTICS

a. BACKFAT THICKNESS, cm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.00534	0.00178	0.13		0.2057
Trt	2	0.07639	0.03819	2.70	0.146	
Residual	6	0.08477	0.01413			
Total	11	0.16650				

b. BELLY, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.22339	0.07446	1.50		0.3857
Trt	2	0.00711	0.00355	0.07	0.932	
Residual	6	0.29810	0.04968			
Total	11	0.52859				

c. CARCASS LENGTH, cm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	2.9727	0.9909	1.59		1.366
Trt	2	1.7682	0.8841	1.42	0.313	
Residual	6	3.7422	0.6237			
Total	11	8.4831				

d. CHILLED DRESSED WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	1.2643	0.4214	1.16		1.042
Trt	2	0.0937	0.0469	0.13	0.881	
Residual	6	2.1771	0.3628			
Total	11	3.5352				

e. EMPTY GIT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.30016	0.10005	6.84		0.2093
Trt	2	0.08010	0.04005	2.74	0.143	
Residual	6	0.08781	0.01464			
Total	11	0.46807				

f. FILLET, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0006891	0.0002297	0.92		0.02739
Trt	2	0.0001885	0.0000943	0.38	0.702	
Residual	6	0.0015031	0.0002505			
Total	11	0.0023807				

g. FULL GIT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0923	0.0308	0.21		0.658
Trt	2	0.1301	0.0651	0.45	0.658	
Residual	6	0.8674	0.1446			
Total	11	1.0898				

h. HEAD, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.05171	0.01724	0.57		0.3020
Trt	2	0.02909	0.01454	0.48	0.642	
Residual	6	0.18279	0.03046			
Total	11	0.26358				

i. HEART, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.00076823	0.00025608	3.47		0.01486
Trt	2	0.00007813	0.00003906	0.53	0.614	
Residual	6	0.00044271	0.00007378			
Total	11	0.00128906				

j. KIDNEY, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0008854	0.0002951	1.21		0.02697
Trt	2	0.0001042	0.0000521	0.21	0.813	
Residual	6	0.0014583	0.0002431			
Total	11	0.0024479				

k. LIVER, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.06504	0.02168		1.51	0.2074
Trt	2	0.07323	0.03661	2.55	0.158	
Residual	6	0.08625	0.01438			
Total	11	0.22452				

l. LIVEWEIGHT AT SLAUGHTER, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.4518	0.1506	0.40		1.057
Trt	2	1.1354	0.5677	1.52	0.292	
Residual	6	2.2396	0.3733			
Total	11	3.8268				

m. LOIN WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.08585	0.02862	0.61		0.3749
Trt	2	0.02599	0.01299	0.28	0.767	
Residual	6	0.28165	0.04694			
Total	11	0.39349				

n. LOIN EYE AREA, cm^2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	12.569	4.190	1.44		2.947
Trt	2	6.396	3.198	1.10	0.391	
Residual	6	17.407	2.901			
Total	11	36.372				

o. P2 MEASUREMENT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.014740	0.004913	0.65		0.1507
Trt	2	0.034803	0.017402	2.29	0.182	
Residual	6	0.045521	0.007587			
Total	11	0.095064				

p. RESPIRATORY TRACT WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.050768	0.016923	8.86		0.0756
Trt	2	0.001354	0.000677	0.35	0.715	
Residual	6	0.011458	0.001910			
Total	11	0.063581				

q. SHOULDER WEIGHT, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.08962	0.02987	1.44		0.2494
Trt	2	0.00176	0.00088	0.04	0.959	
Residual	6	0.12469	0.02078			
Total	11	0.21607				

r. SPLEEN, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0013932	0.0004644	0.62		0.04741
Trt	2	0.0007031	0.0003516	0.47	0.647	
Residual	6	0.0045052	0.0007509			
Total	11	0.0066016				

s. STOMACH (EMPTY), kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.023268	0.007756	3.26		0.0844
Trt	2	0.001354	0.000677	0.28	0.762	
Residual	6	0.014271	0.002378			
Total	11	0.038893				

t. THIGH, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.15162	0.05054	4.14		0.1912
Trt	2	0.03682	0.01841	1.51	0.295	
Residual	6	0.07328	0.01221			
Total	11	0.26172				

u. TROTTERS, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.003893	0.001298	1.16		0.05779
Trt	2	0.000078	0.000039	0.04	0.966	
Residual	6	0.006693	0.001115			
Total	11	0.010664				

v. VISCERA, kg

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.1302	0.0434	0.21		0.791
Trt	2	0.3307	0.1654	0.79	0.495	
Residual	6	1.2526	0.2088			
Total	11	1.7135				

w. WARM DRESSED WEIGHT, kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	2.8229	0.9410	1.78		1.259
Trt	2	0.3229	0.1615	0.30	0.748	
Residual	6	3.1771	0.5295			
Total	11	6.3229				

Table (v). ANALYSIS OF VARIANCE (ANOVA) TABLES FOR MEAN RELATIVE WEIGHTS OF SOME BODY COMPONENTS

a. BELLY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.4506	0.1502	1.40		0.5676
Trt	2	0.0368	0.0184	0.17	0.847	
Residual	6	0.6456	0.1076			
Total	11	1.1330				

b. CHILLED DRESSED WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	3.531	1.177	0.95		1.925
Trt	2	0.406	0.203	0.16	0.852	
Residual	6	7.427	1.238			
Total	11	11.365				

c. EMPTY GIT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.54285	0.18095	6.02	0.194	0.3000
Trt	2	0.13143	0.06571	2.19		
Residual	6	0.18040	0.03007			
Total	11	0.85468				

d. FILLET

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0014266	0.0004755	0.88		0.04014
Trt	2	0.0003463	0.0001732	0.32	0.737	
Residual	6	0.0032293	0.0005382			
Total	11	0.0050023				

e. FULL GIT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.2244	0.0748	0.27		0.918
Trt	2	0.3033	0.1516	0.54	0.609	
Residual	6	1.6880	0.2813			
Total	11	2.2157				

f. HEAD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.14008	0.04669	0.93		0.3875
Trt	2	0.05020	0.02510	0.50	0.630	
Residual	6	0.30100	0.05017			
Total	11	0.49127				

g. HEART WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0015543	0.0005181	4.78		0.01801
Trt	2	0.0000969	0.0000485	0.45	0.659	
Residual	6	0.0006498	0.0001083			
Total	11	0.0023011				

h. KIDNEY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.0017744	0.0005915	1.26		0.03750
Trt	2	0.0002061	0.0001031	0.22	0.809	
Residual	6	0.0028184	0.0004697			
Total	11	0.0047989				

i. LEAF FAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.02568	0.00856	0.73		0.1871
Trt	2	0.03668	0.01834	1.57		0.283
Residual	6	0.07019	0.01170			
Total	11	0.13255				

j. LIVER

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.12273	0.04091	1.59		0.2773
Trt	2	0.14398	0.07199	2.80	0.138	
Residual	6	0.15412	0.02569			
Total	11	0.42083				

k. LOIN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.19602	0.06534	0.92		0.4610
Trt	2	0.10988	0.05494	0.77	0.502	
Residual	6	0.42602	0.07100			
Total	11	0.73192				

l. RESPIRATORY TRACT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.093572	0.031191	8.22		0.1066
Trt	2	0.001034	0.000517	0.14	0.875	
Residual	6	0.022769	0.003795			
Total	11	0.117375				

m. SHOULDER WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.21291	0.07097	1.42		0.3872
Trt	2	0.01479	0.00740	0.15	0.866	
Residual	6	0.30045	0.05008			
Total	11	0.52816				

n. SPLEEN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.002854	0.000951	0.68		0.0647

Trt	2	0.001625	0.000812	0.58	0.588	
Residual	6	0.008388	0.001398			
Total	11	0.012867				

o. STOMACH (EMPTY)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.043661	0.014554	3.09		0.1187
Trt	2	0.002668	0.001334	0.28	0.763	
Residual	6	0.028248	0.004708			
Total	11	0.074577				

p. THIGH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.38587	0.12862	2.96		0.3605
Trt	2	0.02456	0.01228	0.28	0.763	
Residual	6	0.26046	0.04341			
Total	11	0.67090				

q. TROTTERS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.005956	0.001985	0.91		0.0809
Trt	2	0.000483	0.000241	0.11	0.897	
Residual	6	0.013103	0.002184			
Total	11	0.019542				

r. VISCERA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	0.1964	0.0655	0.17		1.089
Trt	2	0.8286	0.4143	1.05	0.408	
Residual	6	2.3782	0.3964			
Total	11	3.4032				

s. WARM DRESSED WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	3	7.351	2.450	1.53		2.189
Trt	2	0.182	0.091	0.06	0.945	
Residual	6	9.607	1.601			
Total	11	17.140				

Table (vi). ANALYSIS OF VARIANCE (ANOVA) TABLES FOR BLOOD PROFILE

a. HAEMATOCRIT, %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	312.99	34.78	2.61		3.428
Trt	2	0.80	0.40	0.03	0.970	
Residual	18	239.59	13.31			
Total	29	553.37				

b. HAEMOGLOBIN, g/dL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	36.321	4.036	2.81		1.126
Trt	2	0.392	0.196	0.14	0.873	
Residual	18	25.835	1.435			
Total	29	62.548				

c. MCH, pg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	673.71	74.86	1.00		8.11
Trt	2	114.35	57.18	0.77	0.479	
Residual	18	1341.41	74.52			
Total	29	2129.47				

d. MCHC, g/dL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	45.405	5.045	0.82		2.324
Trt	2	8.442	4.221	0.69	0.514	
Residual	18	110.125	6.118			
Total	29	163.972				

e. MCV, fL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	130.90	14.54	1.24		3.712
Trt	2	76.37	38.19	3.27	0.062	
Residual	18	210.32	11.68			
Total	29	417.59				

f. MPV, fL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	2.5950	0.2883	0.43		0.769
Trt	2	0.3247	0.1623	0.24	0.787	
Residual	18	12.0620	0.6701			
Total	29	14.9817				

g. PCT, %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	0.059072	0.006564	0.99		0.0764
Trt	2	0.024511	0.012256	1.85	0.185	
Residual	18	0.118930	0.006607			
Total	29	0.202513				

h. PLATELETS, x10/L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	62823.	6980.	0.88		83.6
Trt	2	18792.	9396.	1.19	0.328	
Residual	18	142554.	7920.			
Total	29	224169.				

i. RBC, $x10^{12}/L$

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	6.7162	0.7462	1.59		0.644
Trt	2	0.2616	0.1308	0.28	0.760	
Residual	18	8.4444	0.4691			
Total	29	15.4222				

j. WBC, $x10^{9}/L$

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	LSD
Rep stratum	9	104.49	11.61	0.74		3.729
Trt	2	2.78	1.39	0.09	0.916	
Residual	18	283.51	15.75			
Total	29	390.78				

Table (vii). Haematology reference values for swine[ø].

Haematology parameters	Range
Haemoglobin (g/l)	100-160
Haemogram (µmol/L)	6.2-9.9
Haematocrit/PCV (L/L)	0.32-0.50
Red Blood Cell ($x10^6$/µl)	5-8
Mean Cell Volume (fl)	50-68
Mean Cell Haemoglobin (pg)	17-21
Mean Cell Haemoglobin Concentration (g/L)	300-340
Reticulocytes ($x10^9$/L) [Ψ]	0-80
White Blood Cell ($x10^3$/µl)	11-22
Neutrophils (mature) ($x10^9$/L)	3.1-10.5
Neutrophils (band) ($x10^9$/L)	0-0.9
Lymphocytes ($x10^9$/L)	4.3-13.6
Monocytes ($x10^9$/L)	0.2-2.2
Eosinophils ($x10^9$/L)	0.1-2.4
Basophils ($x10^9$/L)	0-0.4
Platelets ($x10^9$/L)	320-720
Plasma proteins (g/L)	60-80
Fibrinogen (g/L)	1-5

[ø]Source: Blood DC, Studdert VP: Saunders, comprehensive veterinary dictionary, ed. 2, Philadelphia, 1999, WB Saunders, p 1252. Reference values may be influenced by the method of measurement and by the animal's breed, sex, age and environment; hence, these values are guidelines only.

[Ψ]Aggregate reticulocytes derived from Fan LC., Dorner JL., Hoffman WE: J Am Anim. Hosp. Assoc, 14: 219, 1978.

Table (viii). Blood biochemical reference values for swine[ø].

Biochemistry parameters	Range
Albumin (g/L)	27-39
Alkaline phosphatase (U/L)	0-500
Alkaline transaminase (U/L)	-
Amylase (U/L)	-
Aspartate aminotransferase (U/L)	-
Total bilirubin (µmol/L)	0-4
Calcium (mmol/L)	1.80-2.90
Chloride (mmol/L)	99-105
Cholesterol (mmol/L)	2.0-5.0
Creatinine (µmol/L)	90-240
Glucose (mmol/L)	3.6-5.3
Lipase (U/L)	-
Magnesium (mmol/L)	0.8-1.6
Phosphorus (mmol/L)	1.6-3.4
Protein (g/L)	61-81
Potassium (mmol/L)	4.7-7.1
Sodium (mmol/L)	140-150
Urea (mmol/L)	3.0-8.5

[ø]Source: Animal Health Laboratory, Laboratory Services Division, University of Guelph, Ontario, Canada. Reference intervals may vary from laboratory to laboratory and depend on the method used, as well as the animal's breed, sex, age and environment.