TITLE PAGE

EFFECTS OF GARLIC (*Allium sativum*) AND PROBIOTICS (*Lactobacillus acidophilus*) ADDITIVES IN THE DIETS OF GROWER PIGS.

BY

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CERTIFICATION

This work has been certified as original, dully supervised and was carried out by UGWUOKE, JERVAS IKECHUKWU PG/M.SC/08/48569 in the Department of Animal Science of this University.

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DEDICATION

This work is dedicated to my lovely wife, Ebere and my special brother Christ Dike and family.

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I wish to express my gratitude to God for His abundance grace upon my life, without which this study would not have been successful.

Sincerely, I appreciate all advice, attention and close supervision of my supervisor Dr. A. E. Onyimonyi and the fatherly advice from Prof. S.O.C. Ugwu during this study process.

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TO GOD BE THE GLORY!

Jervas

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ABSTRACT

This study which lasted for 12 weeks investigated the effects of garlic and probiotics additives in the diets of grower pigs. A total of sixteen 16 weeks old grower pigs were randomly assigned to four treatments. Each treatment had 2 replicates of 2 pigs per replicate that were housed in a previously cleaned and disinfected pen measuring 3.2m x2.7m with concrete floor, feeding trough and water drinker. Four treatment diets (T1, T2, T3, and T4) were used in a 2x2 factorial arrangement in a Completely Randomized Design. T1 contained 0g garlic and 0g probiotics in a 50kg basal diet. T2 contained 50g of probiotics in a 50kg basal diet. T3 had 50g of garlic in a 50kg basal diet while T4 had 50g of garlic and 50g of probiotics in a 50kg basal diet. Pigs were fed 4% of their average body weight per pen and water was provided ad libitum, while other management principles were observed. The initial body weight (1BWkg), height at withers (HWcm), chest girth (CGcm), body flank (BFcm) and body length (BLcm) were measured and recorded at the beginning of the experiment and subsequently measured bi-weekly till the end of the experiment. At the end of the experiment, 2 pigs were randomly selected from each treatment for carcass, serum and hematological investigation. Blood samples were collected through the retro-bulbar plexus of the medial canthus of the eye of the pigs using syringes and needles and placed in micro tubes with Ethylene diamine tetracetic acid (EDTA) as anti-coagulant. The economic implication of the study was also calculated. Data obtained were subjected to a one-way analysis of variance (ANOVA) using SPSS. Significantly different means were separated using Duncan's New Multiple Range Test. Results obtained indicated that there were statistical differences (P<0.05) in the performance characteristic, linear body measurements, and carcass and organ characteristics. Result on serum biochemistry showed no significant differences (P>0.05) in ALT, AST, TCRE, and Urea among the treatment groups while there were significant differences (P<0.05) in ALP and BIL in T1 but similar in T2, T3 and T4 (P>0.05).

Result on serum cholesterol showed significant differences (P<0.05) in HDL which was similar in T1, T3 and T4. TRIG and VLDL values were also significantly difference (P<0.05) with T1 and T3 being similar but higher than values for T2 and T4 which are themselves similar (P>0.05). LDL values were similar (P>0.05) in T1, T3 and T4 but higher (P<0.05) than T2. Feed cost per kg gain was highly significant (P<0.05) in T1 (220.22±17.61), but similar (P>0.05) in T2 (159.52±10.19), T3 (167.93±13.31) and T4 (151.10±10.94). There were significant differences (P<0.05) in the total white blood cell TWBC with T2 (16.33±1.83), and T3 (16.35±0.45) having comparable means values, while still being highest and lowest in T4 (20.20±0.15) and T1 (12.43±1.63) respectively. However, there were non significant differences (P<0.05) observed in the packed cell volume PCV, red blood count RBC and hemoglobin concentration HbC.

It is concluded that feeding garlic or probiotics to growing pigs results in better performance, serum chemistry and economics of production.

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CHAPTER ONE

INTRODUCTION

1.0 GENERAL

In developing economy, like Nigeria, emphases are placed more on quality, wholesome as well as affordable animal protein for the growing population. The per capita intake of protein, that of animal protein in particular, of the growing population of Nigeria remains very low. This situation is worsening, because other sources of cheap animal protein such as wild animals and other microlivestock such as snails, rats, and grass cutter, etc. are being depleted as a result of deforestation, bush fires, and indiscriminate and uncontrolled hunting (Abeke *et al*., 2009).

In Nigeria as in most developing countries, it has been realized that the development of pig industry is one of the fastest means of bridging the prevailing protein deficiency gap which has developed over the years due to the increasing population. This is undermined by the inadequate supply of their products due, largely, to high cost and inadequate feeding and nutrition, poor breeding stock, and poor management practices involving; proper housing, disease prevention and control.

Aduku (1992) opined that 70 – 90 percent (%) of the cost of production of eggs, chickens, pork, and rabbits have been attributed to feed input. However, the availability of cheap and balanced feed is a key to abundant animal protein production. This is because, feed indicates how many animals a farmer can grow and how fast they can mature for the market.

Pigs, like other non- ruminants compete with man for feed. This is because both categories of consumers depend on the same source for food which supplies are already inadequate. Therefore, in an effort to increase animal protein source, pig production should be encouraged so as to make available pork and bacon to Nigerian meat consumers. This is because according to Serres (1992), pigs are known to be highly prolific and very efficient in converting feed materials into high quality animal protein.

Recent concerns regarding the use of antibiotics as growth stimulating agent in animal production and their residual effects on the part of the consumers has demanded for alternative strategies to improve animal production and health without need for antibiotics. Studies, in recent years, have shown that lactic acid bacteria including *Bacillus spp*. are widely used as probiotics in humans, and their use has reportedly led to health benefits against gastrointestinal disorder; including diarrhea, inflammatory bowel disease, lactose intolerance, and infections (Madson, 2001). Although various *Bacillus spp*. are widely used as probiotics for human and animals, their mechanism of action is not yet fully understood (Hong *et al.*, 2005).

Food and Agricultural Organization (FAO, 2005) defined probiotics as a live microorganism administered in adequate amount which confers a beneficial health effect on the host. Ezema (2007) defined probiotics as life culture of microbes often lactic acid bacteria but also other species such as saccharomyces which are fed to animals to improve their health and growth by altering intestinal microbial balance.

Also, searching for hypolipidemic agent in predominantly vegetation human diet has yielded considerable information concerning the effects of plant materials on cholesterol metabolism in animal models. Garlic (*Allium sativum*), which has been used as spice and folk medicine since antiquity, has been considered to be beneficial to animal health as the allicin, the bioactive substance of garlic has antibacterial, anti parasitic and antifungal activities. The magnitude of this action varies from 14% lowering of serum cholesterol in human to a lowering of 80% in cholesterol fed rabbits (Badia *et al*., 1975; Badia, 1981). Sharma *et al*. (1979) also reported that egg cholesterol was reduced by feeding 1 or 3% garlic powder to laying hens during 3 weeks; Sklan *et al*. (1992) reported that hepatic cholesterol concentration of laying hens was decreased when 20% garlic was fed for 2 weeks. Accordingly, various herbs or their extracts are now being utilized as feed additives due to their widespread anti oxidative and antimicrobial actions, beneficial effects on palatability and gut functions. In order to produce quality and affordable pork for Nigerian consumers, the present study was designed to achieve the under listed objectives.

1.1 Objectives of the Study

The study was designed to achieve the following objectives;

- Determine the effects of garlic and probiotics additives on the growth performance of grower pigs.
- Determine the hematological characteristics of grower pigs' diets containing garlic and probiotics additives.
- Determine the effects of garlic and probiotics additives on the serum chemistry of grower pigs.

1.2 Significance of the Study

A success in providing meat with low cholesterol content will help rekindle the confidence of meat consumers. This is against the background that many meat consumers have reduced the quantity of meat they consume because of the apparent cholesterol content of meat. Literature evidence suggests that garlic and probiotics have ability to reduce cholesterol content of animals. Garlic supplemented diet may inhibit the synthesis of cholesterol and fatty acid in the liver (Yeh and Liu, 2001). Warshafsky *et al*. (1993) suggested that cholesterol lowering of 9% (0.59 mmol/L) could be achieved by a daily consumption of 1.5 – 3g of garlic for 2 – 6 months. Allicin, which causes the characteristic garlic odour, is believed to be the active lipid-lowering compound in garlic.

In the same vein, probiotics have been found to be beneficial to the host gut health, by their antimicrobial activities against gastrointestinal pathogens such as *Salmonella* and *E. Coli,* prevention and treatment of diarrhea in infants and adults, and alteration of the composition and metabolism of the intestinal micro biota (Servin, 2004).

CHAPTER TWO

LITERATURE REVIEW

2.0. PIG PRODUCTION

The domestic pigs *Sus scrofa domesticus* was derived from the wild boars of Europe (*Sus scrofa*) and Asia (*Sus vittatis).* Zoologically, pig is classified according to;

Kingdom	-	*Animalia*
Phylum	-	*Chordata*
Class	-	*Mammalia*
Order	-	*Artiodactyla*
Sub-order	-	*Sus formes*
Family	-	*Suidae*
Genus	-	*Sus*
Species	-	*Scrofa*
Botanical name	-	*Sus scrofa*

(Serres, 1992)

Pig and poultry production has been reported to represent the fastest means of bridging the animal protein supply gap in Nigeria. This is based on the fact that outside their short generation interval, they are known to have rapid fecundity and best efficiency of feed conversion into high quality animal protein (Smith, 2001; Holness, 2005).

Pig is able to eat everything edible to man and other animals including forage because it has 15, 000 taste buds as against 9,000 in human. Moreso, pigs have been shown to digest and utilize fibrous diets (Laswai *et al*., 1997). This is particularly important since the high cost and scarcity of grains and concentrates have been the major constraints to pig production in the tropics.

Studies have also shown that pigs are the most efficient animals that can convert kitchen wastes and other non-conventional feedstuffs into meat (Kyriazakis *et al*., 2006). Their carcasses are easier to dress and have superior curing qualities which are an outstanding advantage for processing and marketing.

Pigs adapt readily to most environmental conditions and are also adaptable to intensified or diversified agriculture. And apart from pork and bacon production, the droppings from pig is very rich in nutrients; can be used in making good fertilizers for crop production and can also be recycled into livestock feeds. Following the ban on the use of antibiotics as growth promoters and the increasing need to increase livestock production, there is then the urgent need to find alternative ways of achieving this, thus, the introduction of garlic and probiotics, both for prophylactic and therapeutic uses (Trafalska *and Grzybowska*, 2004).

2.1. Nutrient Requirement for Pig

Pigs need nutrients for survival and are typically provided with these nutrients as mixed and balanced diet. 60 – 70 percent of production cost is feed cost since diet that is insufficient or excessive in the nutrient of pig lead to unsatisfactory performance, understanding the basic nutritional needs of pig and how to administer them is important (Ensminger *et al.*, 1996).

In determining the appropriate diet for a given pig, the body size, level of productivity, genetic potentials and environmental condition must be considered.

Different nutrient requirement by pigs include:

- Protein

- Energy
- Water
- Vitamins and minerals

Protein

This provides the primary blocks for building the animals body which are meat, collagen, hair and nails. When diets containing protein are taken by the animal, the protein is broken down into amino acids, the actual building blocks. There are about twenty known amino acids, out of which ten are known to be essential because the pig's body cannot synthesize them internally and therefore, must be supplied in the diet. The most limiting of all the amino acids in a practical ration has been recognized to be lysine and methionine. However, their synthetic products are available commercially and can be added in pig rations directly to overcome any deficiencies. Although protein source are available commercially, they are expensive and are always the most limiting in commercial feeds. Common protein containing feedstuffs in Nigeria are: Soybean meal, cotton seed cake, groundnut cake, Soybean (full fat), blood meal, palm kernel cake etc.

The addition of probiotics (lactobacilli) to a practical pig ration with low crude protein content is of great importance. This is because lactobacilli primarily use carbohydrate as growth medium while pathogens use protein. And by decreasing the pathogenic population, more proteins are made available for absorption in the gastrointestinal tract (GIT). Yu *et al.* (2008) demonstrated that *Lactobacillus fermentum* ($5.8x10^7$ $CFUg^{-1}$) maximized the crude protein digestibility among the different concentration of Lactobacillus fermentum in the control diet. Meng (2010) also reported that pigs fed probiotics (mixture of spray – dried spore forming *Bacillus subtilis* and *C.butyricium* *endospores*) showed greater crude protein and energy digestibility compared with those in non –probiotics treatments in growing pigs. Bhandari *et al.* (2010) reported also that probiotics (non –pathogenic *E. coli*, 50ml of $9x10^{10}$ $CFUml^{-1}$ per day) fed with low protein of about 17% diet increased performance of weaning pigs.

Water

Water is the single nutrient required in the greatest quantity by animals. Pigs require water for variety of reasons, including most metabolic functions, adjustment of body temperature, movement of nutrients into the body tissues, removal of metabolic wastes, production of milk and for growth and reproduction.

Pigs consume the majority of the water by drinking. However, some water is ingested in feed and metabolism also generates water. The pig looses its body water through urine, feces, respiration and from the skin. It is necessary to recognize that there is no single water requirement for a particular species or an individual pig; the amount of water consumed depends upon factors such as temperature, diet, frequency with which water is provided, housing and stress in the environment.

Water intake of pigs at various stages

Results of various studies designed to evaluate water intake on performance of growing/finishing pigs are somewhat conflicting. This variation in results is due to the numerous compounding factors involved in the experimental design (i.e. of pigs per pen, ambient temperature, diets etc.) (Barber *et al.* 1989).

Most studies on water consumption by lactating sows have established voluntary intake levels and not the absolute requirements (Barber *et al*., 1989). Reported water intake by lactating sows ranges from 8 to 25 liters (2-6 gallons/sow/day). Litter size (milk demand and production), ambient temperature, and quantity/quality of feed intake can influence water consumption. Conversely, reduced water intake will decrease feed intake, milk production and pig performance (Fraser *et al*., 1990).Water intake by gestating sows was reported to be between 2-5gallons/sow/day. Although, limited information is available on the potential influence of water delivery system on the health of gestation sows (Nienaber and Halu, 1984). As mentioned by Fraser *et al* (1990) research on water requirements of pigs needs to look well beyond "average" values so that the animals do not become dehydrated or affected by cystitis. A summary of water requirement of pigs are provided in Table 1. And these values are based on the requirements of pigs in a thermo neutral environment and under ideal conditions.

Table1. **Water requirement of pigs**. Values (liters/day or gallons/day) indicate the range of requirements as presented in the literature

Class of pigs	Litter/pig/day	Gallons/pig/day
Nursery pigs	2.8	0.7
(up to 60 Ibs BW)	2.5 – 3.0 L/kg of feed consumed	0.3 gal/Ib of feed consumed
Grower pigs	8 – 12	2.3
(60 – 100 Ibs BW)	2.5 – 3.0 L/kg of feed consumed	0.3 gal/Ib of feed consumed
Finishing pigs	12 – 20	3.5
(100 – 250 Ibs BW)	2.5 – 3.0 L/kg of feed consumed	0.3 gal/Ib of feed consumed
Non pregnant gilts	12	3
Pregnant sows	12 – 25	3 – 6
Lactating sows	10 – 30	2.5 – 7
Boars	20	5

Adopted from Lewis and Southern (2001).

Factors affecting water requirements and intake.

Pigs affected with disease require more water than healthy pigs of the same age and body weight. For example, water loss associated with diarrhea or increased water demands of an animal with a fever change the water requirement of a sick pig.

Water demand will increase in proportion with the crude protein content of the diet. Thus, 3.9 and 5.3 liters of water were consumed daily by piglets fed 12 or 16% crude protein diets respectively (Brooks and Carpenter, 1990). The influence of added artificial lysine to the diet on water intake has not been addressed but pigs consuming a pelleted ration have greater water demand than pigs eating a diet fed as meal. High salt or potassium intake increases the demand for water. “Salt poisoning” is not generally a result of a toxic level of salt intake per se, but a disruption of the pigs water balance (i.e. a disruption of water supply).

High ambient temperature will increase water requirements, particularly in sows and finishing pigs. The increased consumption coupled with increased urinary water loss is an effective mechanism by which pigs lose body heat.

Lack of water leads to rise in body temperature and death in pig. And sub-optical amount of water will have a major effect on food intake and pig performance.

Water deprivation in pigs has the following symptoms:

- Reduced feed intake
- Tail biting
- Diarrhea in piglets
- Crowding around water
- Pigs become agitated and irritable
- Increased heart rate and body temperature

The addition of probiotics and garlic in pig ration seem to have increased water intake on growing pigs. Although this has not been fully established and published, it is postulated that the increase in water intake is due, largely, to the protein sparing effects of probiotics in the GIT of the pigs and the form in which the feed was administered (Chang *et al.,* 2001).

Vitamins and Minerals

These are required by pigs and most feed suppliers offer vitamins and trace minerals mixes that have been formulated to meet the requirement of pigs at different stages of growth. Storage of vitamins and mineral premixes should be given careful attention to prevent loss of their effectiveness especially when not stored in accordance with the manufacturer's instructions.

Vitamin needs of swine are met either from that contained in the feed or from synthesis in the body of the animal. Vitamins of particular attention in pig production include the following: A, D, E, riboflavin, niacin, pantothenic acid, choline and B12. In addition to these vitamins, vitamin K, vitamin C, biotin and folic acid are needed in adequate amounts. Recent research indicated that sows receiving supplemented folic acid have larger litters farrowed (John and Trygve , 2011).

Vitamin A function in the growth of both skeletal and soft tissues of the body: in vision, reproduction and disease resistance. Vitamin A does not occur in plant products but the plant pigments carotene can be converted to vitamin A in the intestinal wall of the pig.

Vitamin D functions in the pig's body to increase the absorption of calcium and phosphorus from the intestine and is very necessary for bone growth and calcification.

Vitamin E is required for normal reproduction and growth. Common swine feeds are good sources, green pasture, cured hay, and whole grain and germ parts of grain. Vitamin E deficiencies are not common, but as a safeguard, it can be added cheaply in premix.

Vitamin K is supplied in adequate amount to swine diets to play its blood clotting role.

The table below shows the Missouri recommended vitamin addition per ton of feed (growing- finishing pigs).

Table 2.Vitamin Requirements of Pigs

Nutrient	Unit	Addition per ton	
		Grower	Finisher
Vitamin A	Mil.iu	5.0	3.0
Vitamin D	Thou .iu	500.0	300.0
Vitamin E	Thou.iu	20.0	10.0
Vitamin K	G	2.0	1.0
Riboflavin	G	4.0	2.0
Niacin	G	30.0	18.0
Pantothenic acid	G	16.0	8.0
Choline	G	200.0	100.0
Vitamin Biz	mg	25.0	12.5

Source; John and Trygve, (2011).

Macro minerals of importance to pig are: calcium, phosphorus, and sodium. Calcium and phosphorus are linked as major components of bone and are deposited in the ratio of 2:1. Their availability depends on the feed source, and mineral status of the body.

In general, the ratio of dietary calcium to available phosphorus should not exceed 2:1 otherwise, toxicity may occur.

Sodium is usually provided as common salt which is widely used in the diet at the level of 0.3%. Although not expensive, dietary level of common salt above 1% may cause salt toxicity, especially where water is restricted, and should be avoided (Lewis and Southern, 2001).

Phosphorus is typically stored in an inaccessible form – phytase, but is found in many feeds. Pigs do not have the enzyme phytase and therefore, cannot use much of the phosphorus found in grains. Including synthetic phytase in pig diets allows pig to utilize the phosphorus naturally found in feed and in turn reduces the need for including phosphorus supplement, while also reducing phosphorus excretion.

Energy

Pigs need energy to grow and the energy requirement of pig is usually given in terms of digestible or metabolic energy which is measured in kilo calories (Kcal) or Mega joules (Mj).The bulk of energy in pig ration is supplied by carbohydrates which are the major components of cereal grains such as maize, millet, guinea corn, and root crops such as yam, cassava and potatoes. Fats also contain energy of higher levels than that of carbohydrate. And fats commonly used in pig rations include palm oil, groundnut oil, soy bean oil, and tallow. In pig production, the energy concentration of a diet determine feed intake. Hence, pigs eat feed until they have consumed adequate energy to meet their daily needs. And when pigs are fed low energy diets, their gut capacity may limit growth. Although not typical for producers feeding corn, soybean meal diets, but might become a problem when feeding forage to growing pigs.

When growing pigs were fed probiotics (mixture of spray- dried spore forming *Bacillus subtilis* and *C-butyricuim endospores*) they showed greater energy digestibility compared with those on non probiotics treatments (Meng *et al* (2010). Because of the importance of energy intake in driving average daily gain and market weight, high energy diets can often increase margin over feed cost and, thus, net profit, while not being the lowest in feed cost per kg of gain. This is particularly applicable to young pigs, up to about 60kg body weight and finishing pigs that are managed under practical condition; that are crowded or that are under mild heat stress.

2.2. Blood Composition of Pig:

The blood of pig is a bright viscous fluid that is slightly alkaline to taste with a smell peculiar to it. It is composed of a fluid portion, the plasma which floats three kinds of cell; red cells or erythrocytes, white cell or leucocytes and the blood platelets or thrombocytes. Generally, blood functions as a transport medium, and is heavier and three times as viscous as water. During circulation, it carries with it water and dissolved nutrients to cells and removes waste products, replacing constantly, carbon dioxide in the lungs with oxygen, distribute hormones, circulate antibodies and heal wounds through its coagulatory properties (Frandson, 1986).

2.3. Erythrocytes

Are small biconcave discs that have no nuclei which are the most frequent cells in the blood forming about 45% of its total volume (Woodliff and Herman, 1978). Erythrocyte values include the red blood cells, the hemoglobin content; packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). Hemoglobin constitute about 90-95% of the total solid in the blood cells. Hemoglobin gives red blood cells their property of carrying oxygen and aiding in the transport of carbon dioxide and other internal secretions and nutrients like hormones, glucose and other metabolic products (Swenson and Reece, 1993). The total red blood cells are measured in millions per mm^2. Talbot and Swenson (1993) reported the average number of circulating red blood cells of 4.8 ± 0.9 million per mm^2 at 6-18hrs of age and 6.0 ±1.3 million per mm^2 of blood at 8 weeks in growing pigs. Miller *et al* (1961) also reported a red blood cell count of 4.5 million per mm^2 on the lowest value and 7.6 million per mm^2 on higher value for mature pig. The packed cell volume (PCV) measures the column of packed red cells in percentage when blood is centrifuged at specific revolution per minute (rpm) over a period of time. Ramirez *at al* (1963) after working with 169 pigs reported a high PCV value of 36% at birth, 25.4% at 2 days, 35.4% at 2 weeks and 33.19% at 5 weeks. While Miller *at al* (1961) observed a range of value from 30-44% for pigs from birth to 2 years old. Vaiman *et al* (1968) obtained PCV values of 31.3% at birth, 32.5% at 35days, and 42% at 5 – 7 months. Grupta (1973) showed an average value of 51.5% for adult pigs. Schalm *et al* (1975) gave an average range of PCV values between 37.8 – 40.6% for growing to fishing pigs. Hawkey *et al* (1994) reported that if there are too little red blood cells in the blood per unit volume, It might suggest anemia, a disease where the body does not have sufficient red cells while otherwise may suggest polycythemia. Aengwanich and Tanomtong (2007) reported that red blood cell count is considerably lesser in female (as a consequence of androgen) than their male counterpart. Campbell and Coles (1986) opined that oestrogen decreases count, while androgen and thyroxin have erythropoietic effects.

2.4. Erythrocyte Indices

These include the mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). The (MCHC) measures the cells in micrograms whereas the mean corpuscular volume measures the volume of a single erythrocyte in cubic microns (Wikipedia, 2009).

2.5. Haemoglobin Content

This is the protein that constitute up to about 90% of the solids in red blood counts. It is characterized by its ability to combine with the oxygen in the lung to unstable oxy-haemoglobin and gives the blood its bright red colour. Without oxygen, haemoglobin appear dark blue and when heamoglobin is librated by the corpuscles, the blood is said to have undergone heamolysis or laked (Jain, 1993). The heamoglobin counts of males are usually higher than that of females due to the peculiarity of hormones present in the two sexes (Campbel, 1995). Heamoglobin content is measured in grammes per 100ml of blood.

2.6. Leukocyte Values

The leukocytes do not have pigments and are usually larger than the erythrocyte while containing nuclei. Their primary function is provision of defense of the body against infection. According to Woodliff and Herrman (1978) they form less than 1% of the blood volume. Scientifically, pigs have been proved to generally have high white blood cells of 10,000 to 15,000 per mm^3 of blood for piglets and 15,000 to 20,000 per mm^3 of blood for growing pigs. There are three main groups of leucocytes which are; the lymphocytes, the monocytes and the granulocytes. The granulocytes have specific granules in their cytoplasm and are differentiated into classes according to their staining characteristics and the eosinophils which are acid staining in character (Swenson and Reece, 1993)

The lymphocytes and neutrophils are active phagocytic and the eosinophils are responsible for the detoxification of protein breakdown products (Bentinck Smith, 1969). Hemoglobin concentration is expressed in g/100cm^3 while MCHC is expressed in microgrammess; total WBC in thousands per mm^3.

Table 3. Normal Hematological Values of Pigs.

Blood Parameters	Range	units
PCV	32-47	%
Hb	9.0-16.8	g/dl
RBC	5.0-9.0	x10^6/ul
MCV	50-62	%
MCHC	31-34	%
MCH	16-19	%
WBC	8.6-20	mm^3

Source: Coffin (1957)

2.7. Definition of Probiotics.

There have been several definitions of probiotics over the years. Hataka *et al,* (2002) defined probiotics as naturally occurring micro-organism that function internally to promote healthy digestion, boost immune system and contribute to the general wellbeing of the animal. Schrezenemier and De Verse (2001) defined probiotics as viable microbial food supplements which beneficially influence the health of the host." The most recent definition was by Weichselbaum (2009) that probiotics are live microorganisms which have been found to confer a health benefit on the host when administered in adequate amount.

Probiotics are mainly used to reinforce or re-establish the gut microbial balance, especially when the hosts are confronted with challenges or stress (Vanabelle, 2001). Some studies have suggested that administration with different microbes in early life can alter the composition of gut flora during the first weeks of life and have an impact on health in later life (Bjorksten *et al*., 2001; Kero *et al*., 2002). It is common to supplement lactic acid bacteria (LAB) probiotics as they are considered as natural microflora of the gut. Examples of probiotic organisms are;

1. Lactobacilli e.g. (i) Acidophillus

(ii) Streptococcus

(iii) Pediococcus

(iv) Bifidobacteria

2. Yeast .eg. *(i) Saccharomyces bourdallis*

(ii) Saccharomyces cerevisae.

2.7.1. Species of Probiotics:

Many strains of bacteria have been used as probiotics, the most commonly used species being lactic acid bacteria such as *lactobacillus, Streptococcus* and *Bifidobacteria* (Dunne *et al*., 1999). Commercial species of probiotics are usually isolated from the intestinal microflora of the intended consumer (for example human, chicken or pig) and selected on the basis of criteria such as resistance to stomach acids and bile salts, ability to colonize in the intestine or antagonism of potentially pathogenic micro-organisms.

Lactic acid bacteria are found in large numbers in the guts of healthy animal and do not appear to affect them adversely. According to Chen *et al*. (2006), they were generally regarded as safe for the host. Lactic acid bacteria used as probiotics have included *L.acidiophilus, L.casei, L.delbrueckii, E.faecium, S.diacetylactis, S.intermodus, B.adolescents, B.animalis, B.ifantis, B.longum, L.lactobacilli, Steptococci, B. Bifidobcteria . (L=lactobacilli, S=streptococci, B=bifidobacteria, E=Enterococcus) (Viet, 2006)*. Species other than lactic acid bacteria which are currently being used in probiotic preparations include *Bacillus* species and yeasts (*Saccharomyces cereviciae and Aspergillus oryzae*. *Bacillus* species are mostly soil organism, some of which are used for the production of antibiotic substances and are not normal components of the indigenous microflora (Johnson *et al*., 1992). *Bacillus* products could compete with other intestinal microflora for nutrients (Freter, 1992) or might produce an antibacterial substance (Hentges, 1992) if the product were continually fed. Enzymes, vitamins and other nutrients contained within yeast have been proposed to produce beneficial performance responses in pigs (Kornegay and Riseley, 1996).

2.7.2. Mode of Action of Probotics;

Probiotics function in several ways in the gut when administered orally to the host and the involved mechanisms may diverge due to the different types available. Generally, the modes of action of probiotics are suggested as follows:

i. Producing compounds that are toxic to the pathogens.
ii. Stimulating the immune system.
iii. Competing with pathogens for binding sites on the intestinal epithelium.
iv. Competing with pathogenic bacteria for nutrients in the gut.

i. Producing compounds that are toxic to the pathogens;

Probiotic bacteria produce a variety of substances that are inhibitory to both gram – positive and gram- negative bacteria in the gut which include organic acids, antioxidants and bacteriocins and may reduce not only the number of viable pathogenic organism but may also affect bacterial metabolism and toxin production (Corcionivoschi *et al*., 2010). The bacteriocins produced by lactic acid bacteria have been reported to be able to permeate the outer membrane of the gram – negative bacteria and subsequently induce the inactivation of gram – negative bacteria in conjunction with other enhancing antimicrobial environmental factors such as low temperature, organic acids and detergents (Alakomi *et al*., 2000).

ii. Stimulating the immune system:

Analysis and research into the ability of probiotics to influence immune system in animals and humans is a recent development in this field. Probiotics provide defense to the cells by inducing anti-inflammatory cytokines and reducing pro inflammatory cytokines from enterocytes and intestinal immune cells recruited to sites of inflammation (Wang *et al*., 2009). Some probiotics strains such as *Lactobacillus* have proven to be capable of acting as immunomodulators by enhancing macrophage activities, increasing the local antibody levels, inducing the production of interferon, and activating killer cells (De Simone *et al*., 1993). However, it is difficult to completely conclude that probiotics contribute significantly to the immune system of the host. The main reason behind this caveat is that probiotics differ from antibiotics in that they are not intended to eradicate invasive pathogens in the gastrointestinal tract (G I T). Therefore, such observed improvements or positive effects are always somewhat compromised due to the animals' immune system status and the various applied situations.

iii. Competing with pathogens for binding sites on the intestinal epithelium:

Colonization by a bacteria species is defined by the presence of a bacterial population in the gastrointestinal tract which is stable in size and occurrence over time without the need for periodic reintroduction of the bacteria by repeated oral doses or other means.

The mechanism of colonization is suggested to be associated with certain species within the micro-flora which can influence the expression of glycoconjugates on epithelial cells that may serve as receptors for the adhesion of bacteria. Supported commonly, most intestinal pathogens must adhere to the intestinal epithelium if they are to colonize in the intestine and produce diseases. Consequently, some bacterial strains have been chosen as probiotics for their ability to adhere to the gut epithelium and thus compete with pathogens for adhesion receptors. So probiotics have the competitive exclusion advantage, which is defined by the ability of normal micro-flora to protect against the harmful establishment of pathogens (Jeffrey, 1999).

Attempts to select an individual microorganism or a specific mixture of microorganisms with the specificity of normal micro-flora to resist pathogen invasion have not been successfully established. In order words, an expected effect of the addition of probiotics to the gastrointestinal tract is an increase in normal micro-flora colonization with inhibition of the adhesion of harmful pathogens on the intestinal epithelium (Savage, 1969).

iv. Competing with pathogens for nutrients in the gut:

Probiotics may compete for nutrients and absorption sites with pathogenic bacteria. The gut is such a rich source of nutrients that it may seem unlikely that microorganisms could not find sufficient food for growth (Malago and Koninkx, 2011).

However, it should be noted that the environment only has to be deficient in one essential nutrient in order to inhibit microbial growth. In addition, the ability to rapidly utilize an energy source may reduce the log phase of bacterial growth and make it impossible for the organism to resist the flushing effect exerted by peristalsis.

2.7.3 Benefits of Probiotics:

Researches have shown that there are significant amount of potentials for the application of probiotics in the livestock feed industries. These may induce:

i. Growth promotion of farm animal.
ii. Increased egg production in poultry.
iii. Protein sparing effect.
iv. Nutrient synthesis and bioavailability.

i. Growth promotion of farm animals:

Chang *et al*. (2001) and Ezema (2007) observed that carbohydrates are broken by probiotics bacteria which mean that the food is being split into its most basic elements and makes for increased absorption of nutrients through the digestive system. *Bifidiobacteria* and *lactobacillus* increased weight gain and reduced mortality in piglets. In addition, piglets fed *Bacillus* coagulants had lower mortality and improved weight gain than piglets on unsupplemented diets and did better than piglets fed sub therapeutic antibiotics (Chang *et al*., 2001).

ii. **Increased egg production in poultry:**

During late laying period, supplementation of probiotics increased production, reduced mortality, improved digestibility and feed conversion efficiency but did not change egg quality (Yoruk, 2004).

iii. Protein sparing effects:

Lactobacilli primarily used carbohydrates as growth medium, while pathogens use proteins. However, by decreasing the pathogenic population, more protein is made available for absorption by the host animal (Chang et al., 2001).

iv. Nutrient synthesis and bioavailability:

Probiotics bacteria synthesize certain amino acids which are assimilated directly e.g. Lysine is synthesized from specific strain of *Lactobacillus plantarum*. Probiotics also produces B-Vitamins which include Riboflavin, Niacin, folic acid, B_{12}, B_6 and pantothenic acid which are biocatalyst in food metabolism and in fighting stress (FAO/WHO, 2005).

2.7.4. Some Limitations in the use of Probiotics

i. The effects of probiotics disappear after ceasation of dosing. Chicks fed probiotics supplemented feed failed to show evidence of colonization of the microbes used as probiotics after seven days of withdrawal of feed. Thus showing that the strain introduced can only colonize the gut as long as more adaptable organism is not available (Tannock *et al*., 2003)

ii. Type of probiotics prepared, and the host animals;

Every probiotic has a suitable animal that it can exert its effects on e.g. *S. Cereviasae* is more effective in poultry than human or other animals (Fuller, 1989).

2.7.5 Qualities of an Ideal Probiotics

i. It must produce beneficial effect on the host animal.
ii. It should have the ability to grow and survive in the intestine.
iii. It should be capable of being prepared as a viable product on industrial scale.

iv. It should be stable and viable for a long time period under storage and field conditions.

2.7.6 The Effects of Probiotics in Pigs

i. Growth performance: In the livestock industry, the use of probiotics aims to improve intestinal health which can then lead to better general health and productivity among animals. Inclusion of *Bacillus* species has resulted in improved growth rates and feed efficiency in piglets and grower pigs (Kyriakis *et al.,* 2004). Davis *et al*. (2008) reported that the addition of 0.05% of diet (based on *B. Lichenformis* and *B. Subtilis*; 1.47 x 10^8 CFU) improved average daily gain (ADG) and reduced mortality rates of growing and finishing pigs. Some reports have indicated that feeding lactic acid bacteria by *Lactobacilli* improves performance in suckling pigs, and weaning pigs (Jassek *et al,* 1992), grower pigs (Baird, 1977) and finishing pigs (Hong *et al*., 2002). Giang et al (2010) fed lactic acid bacteria complexes comprising combinations of *Enterococcus feacium* 6H2(3X108CFUg^{-1}), *Lactobacillus acidophilus* C3(4X106CFUg^{-1}) *Pediococcus pentosaceus* D7(3X106CFUg^{-1}), *L. plantarum* 1K8(2X106CFUg^{-1}), and *L. plantarum* 3K2(7x106CFUg^{-1}) to piglets and observed that daily feed intake, weight gain, and feed conversion ratio were improved ($P<0.05$).

ii. Digestibility: Regarding nutrient digestibility as influenced by probiotics, positive effects on performance were observed by Hong *et al* (2002) that probiotics possess a high fermentative activity and stimulate digestion. *Lactobacilli are* known to produce lactic acid and photolytic enzymes which can enhance nutrient digestion in the gastrointestinal tract (Yu *et al*., 2008).

iii. Immunity: Probiotic stimulation of immune system in pigs was observed by several authors (Takahashi *et al.*, 1998; Franscico *et al.*, 1995). The immune modulatory effects might even be achieved with dead probiotic bacteria or just probiotics derived components like peptidoglycan fragments or DNA. Taras (2004) investigated the effects of long-term application of *E. faecium* on performance, health characteristics of sows and offspring and reported that probiotics inclusion reduced overall pre-weaning mortality (16.2 vs. 22.3%) and the rate of piglets with post weaning diarrhea (21 vs. 38%). According to (Yu *et al*., 2008), *Lactobacilli* can colonize and adhere to the gastrointestinal tract epithelium forming a protective membrane against pathogenic microorganisms while at the same time modulate immunity with stimulating epithelial lymphocytes.

iv. Efficacy of probiotics: Under different conditions the efficacy of probiotics may be due to the probiotic preparation itself or various other factors. These factors may include the low survival rate of strains, varying stability of strains, low probiotics doses, frequency/infrequency of probiotics administration, interaction with some medicine (antibiotics and antimicrobials), health and nutritional status of the animal and the effect of age, stress, genetics and type of animals (Bomba *et al*., 2007).

However, research points to the fact that probiotics are most effective in animal during micro-flora development or when micro-flora stability is impaired (Stavric and Kornegay, 1995).

2.8. Garlic (*Allium sativum*)

This is a species in the Onion genus, *Allium*. Its close relatives include the shallot, lack, chive, onions and rakkyo (Block, 2010). Garlic has been used throughout history for both culinary and medicinal purposes. The most commonly used part of the plant is the bulb. With the exception of the single closed types, the bulb is divided into numerous fleshy section called chores which are consumed (raw or cooked) or for medicinal purposes, and have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking (Gernot, 2005).

The binomial classification of garlic can be shown below

Kingdom:	*Plantae*
Phylum:	*Angiosperms*
Subphylum:	*Monocots*
Order:	*Asparagales*
Family:	*Amaryllidaceae*
Subfamily:	*Allioideae*
Genus:	*Allium*
Species:	*Sativum*
Botanical names:	*Allium sativum*

According to Sohary and Hopt (2000), 'A difficulty in the identification of its wild progenitor is the sterility of the cultivars, though it is thought to be descendant from the species *Allium longiscopis,* which grows wild in central and southern Asia. *Allium sativum* grows in the wild in areas where it has become naturalized. The "wild garlic" "crow garlic" and "field garlic" of Britain are members of the species *Allium ursinum, Allium vineale*, and *Allium oleraccum*, respectively.

In North America, *Allium vineale* (wild garlic) and *Allium canadense* (Meadow garlic) are common weeds in fields (McGee, 2004).

Table 4: Nutritive value per 100g of garlic

Energy	623kj (149cal)
Carbohydrate/Sugar	33.06g/1.00g
Dietary fibre	2.1g
Fat	0.5g
Protein	6.39g
Vitamins	34.049g
Minerals	0.782g

Source USDA Nutrient Database (2008)

2.8.1 Properties of Garlic

Garlic (*Allium sativum*) has the following properties

Anthelmintic effects: garlic extracts have been shown to have anthelmentic activities against common intestinal parasites, including *Ascaris lumbricordes* (roundworm) and hook worn (Pena *et al.,* 1988)

Antiviral effects: these have been demonstrated by its protection against mice from infection with intranasally–inoculated influenza virus, and by its enhancement of neutralizing antibody production when given with influenza vaccine (Paul, 1996).

Antifungal effect: Garlic has a very long folk history of use in a wide range of ailments, particularly such as ringworm. Garlic has proved to be effective against infective fungi (Upadhyay, 1980).

Antibacterial effects: studies have shown that the active ingredient of garlic (allicin) inhibited the growth of *Staphylococcus, Streptococcus*, *Bacillus, Brucella,* and *Vibrio* species at low concentrations. Amagase and Millar (1993) also demonstrated its efficacy in inhibiting the growth of some bacteria which had become resistant to one or more of the antibiotics.

Antibiotic/Antimicrobial/Antiseptic effects: *Allium sativum L* "garlic" is a natural antibiotic. And quite unlike other antibiotics, garlic will not deplete the body of flora. It has been shown to have broad-spectrum antimicrobial activities against many genera of bacteria, viruses, fungi and worms. Hence, supporting the historical use of garlic in the treatment of a variety of infectious conditions (Meng and Shyu, 1990; Wargovich *et al.,* 1988). Also, the juice that is obtained from garlic is externally used as an excellent antiseptic for the treatment of wounds (Adetumbi *et al*., 1986).

Hypolipidemic effects: hypolipidemia induced by an excessive ingestion of lipid produces an increase in the plasmatic fibrinogen concentration with a resultant decrease in the fibrinolytic activity, and time of coagulation can be demolished by garlic. The use of garlic has also resulted in a considerable reduction in triglyceride levels due to the production of a marked inhibition in the main lepogenetic enzymes (acetyl COA carboxylase and fatty acid syntheses (Bardia *et al*., 1975; Alder and Holub, 1997; Yeh and Liu, 2001).

Digestive effects: garlic increases bile production while enhancing digestion and reducing stomach gases. It is also carminative and antispasmodic as well as a digestant making it useful in cases of flatulence, nausea, vomiting, colic and indigestion (Rothenburg, 1976).

2.8.2 Medicinal and Health Benefit of Garlic

In vitro studies have shown that garlic has antiviral, antifungal and antibacterial activities which are less clear *in vivo*. Garlic has also been reported to help in the prevention of heart disease (including high cholesterol, high blood pressure, and atherosclerosis), and cancer. Animal studies have suggested possible cardiovascular benefits of garlic. Sovova and Sova (2004) reported that garlic feeding reduced accumulation of cholesterol on the vascular walls of animals. Durak *et al*. (2002) also reported that garlic feeding reduced aortic plaque deposits of cholesterol fed rabbits. Garlic has also been found to provide some help for persons with hyper lipidaemia.

The cholesterol lowering action of garlic is attributed to the allicin content. However, garlic powder, which has low allicin yield failed to show any lipid lowering effect probably because of the different spices preparations (spice mixture) used in the study (Luley *et al*., 1986). Studies have also shown that dietary garlic inhibit the synthesis of lipids in the liver and increases the levels of serum insulin, thereby increasing glycogen in the liver and lowering serum glucose (Chang *et al.,* 1980).
Garlic can help in the control of pathogens, especially bacteria and fungi, and increase the welfare of fish (Corzo – Martinez *et al*., 2007; Adetumbi *et al*., 1986; Ress *et al*., 1993).

Garlic, *Alliums sativa L.,* has been used for the treatment of many diseases since ancient times as reported in the codex Ebers (1550 BC), where an Egyptian medical papyrus described several therapeutic formulas based on garlic as a useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumors (Block 1985).

Generally, many beneficial health properties of garlic are attributed to organosulphur compound, particularly to thiosulfinate (Block 1992). Allicin (Dially thiosulfinate) is the most abundant compound representing about 70% of all thiosulfinates present, or formed in crushed garlic (Block 1992; Han *et al*, 1995). A study which was published in the American Society for Microbiology's *Antimicrobial Agent and Chemotera* reported that allicin in garlic disables the amoebas that cause dysentery by blocking the action of two groups of enzymes – cysteine proteinases and alcohol dehydrogenases. The former enzymes playing a big part in infections by providing the harmful organisms with the means to invade tissues and damage them, while the later enzymes play vital role in the metabolism and overall survival of these organism. However, this is achieved when allicin reacts with sulfhydryl (SH) groups, or thiols, which is an important component of the enzymes (American Society for Microbiology 2009).

2.8.3 Effects of Garlic on Serum Cholesterol

Garlic has unique thisulfinates that condition antithrombotic benefits, including reduced serum cholesterol (Bakhsh and Khan, 1990). Several studies have also shown that garlic is able to diminish blood levels of total cholesterol mainly the most pernicious type that is bounded with low density lipoproteins (LDL). This reduction can be explained by the inhibition of cholesterol synthesis in the liver, since it is able to inhibit enzymes essential for this process (β – hydroxyl-b-methylglutaryl CoA synthesis and b-hydroxyl-b-methylglutaryl CoA reductase). Qureshi *et al*. (1983) reported that serum cholesterol concentrations decreased with increasing levels of garlic powder. They further explained that this may be due to the inhibition of fatty acid synthesis, organic tellurium compounds, and allicin found in garlic that might contribute to lower serum cholesterol by inhibiting squalene epoxidase that is needed in the synthetic pathway of cholesterol.

2.8.4 Effects of garlic additive on pigs.

Results of the study carried out by Yan *et al.* (2011) revealed that when 96 finishing pigs were fed dietary treatments: TI (negative control with high –nutrient-density diet), T2 (positive control with low nutrient –density – diet), T3(t2 +2g/kg fermented garlic powder) for 12 weeks, the average daily gain (ADG) in T3 was higher (P<0.05) than other groups as well as the average daily feed intake (ADFI) during 0-6 weeks. During 6-12 weeks pigs in T3 increased in their ADG greatly (P<0.05) compared to those in T1 and T2.

Wang *et al* (2010) also observed that when rearing pigs were placed on four treatment diets T1 (control basal diet), T2 (control +0.05% fermented garlic), T3 (control+0.010% fermented garlic) and T4 (control +0.20% of fermented garlic) for 5 weeks, the ADFI was higher (P<0.05) in T2 group than in the other treatment group during week 3 to 5 (P<0.05) in response to fermented garlic powder treatments. While only the ADFI in T2 group was increased (P<0.05), overall (0-5wks), T2 treatment increased the ADG by 10% and the ADFI by 12% (P<0.05) when compared with the control group.

The RBC and lymphocyte levels were also reported to be greater (P<0.05) in the T3 and T4 treatment groups at the end of the trial. This inclusion, opines that the addition of fermented garlic powder improved the average daily gain (ADG) in weaning pigs and partially benefited their immunity. There is literature evidence that garlic administration enhanced villus height and crypt dept and decreased epithelial thickness and goblet cell number in duodenum, jejenium and ileum of birds (Adimorabi *et al*., 2006). These morphological changes may have taken place in the guts of the pigs resulting in better feed utilization thus ensuring that nutrients are properly mobilized for increased weight gain. This is in agreement with the views of Ramakrishma *et al* (2003) that garlic additive probably enhanced the activities of pancreatic enzymes and provided micro-environment for better utilization of nutrients.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Location and duration of experiment.

The study was carried out at the Piggery Unit, Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka lies in the derived savanna Region, and is located on longitudes $6^0$25'N and latitude $7^0$24'E (Ofomata, 1975) at an altitude of 430m above sea level (Breinholt *et al*, 1981). Nsukka has a climate of a humid tropical setting with a relative humidity range of 56.01 – 103.83%. Average diurnal minimum temperature range is 22^0C – 24.7^0C, while the average maximum temperature ranges from 33^0C – 37^0C (Energy Centre, UNN, 2008). Two seasons characterize the study area; a rainy season which extends from April-October and a dry season which spans from November-March with no clear cut demarcation. Annual rainfall ranges from 1680mm – 1700mm (Breinholt *et al*, 1981). The entire study lasted for 12 weeks spanning from January-April, 2012.

3.2. Acquisition of experimental animals

A total of sixteen, 16week old grower pigs were randomly selected from the Piggery Unit in the Department of Animal Science Farm, University of Nigeria, Nsukka, for this study.

3.3. Procurement of Experimental Materials.

Probiotics (*lactobacillus acidophilus*) sample was procured from the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Powdered garlic (*Allium sativum*) sample was bought from Ogige Main Market, Nsukka. Feed ingredients were bought and compounded into basal diet from Chidera

Feed Mills using the experimental diets and their percentage composition as shown in Table 5. Canvass tape calibrated in centimeters, spring weighing balance, ruler and jute bags were bought from Ogige Main Market, Nsukka. An electronic weighing scale was provided by the Physiology Laboratory, Department of Animal Science, University of Nigeria, Nsukka.

Table 5: Percentage composition of the basal diet

Ingredients		Level of inclusion (%)
Wheat offal	-	25.0
Palm kernel cake	-	55.0
Groundnut cake	-	15.0
Lime stone	-	2.0
Bone meal	-	2.5
Lysine	-	0.1
Methionine	-	0.1
Salt	-	0.2
Enzyme + Toxin binder	-	0.1
Total	**-**	**100**
Calculated		
Crude protein	-	18.00
Energy (Kcal/kgME)	-	2600

Table 6: Proximate nutrient composition of the basal diet (on as fed basis)

Nutrient	% composition
Moisture	12.20
Crude protein (CP)	18.78
Ether extract (EE)	1.60
Crude fibre (CF)	16.09
Nitrogen free extract (NFE)	44.63
Ash	6.70

3.4. Management of Experimental Animals

Two pigs were randomly assigned to an open sided fly-proof pen measuring 3.2m x 2.75m with concrete floor, feeding trough and water drinker. One (1) of such pens constituted a replicate and two (2) of such replicates constituted a treatment with four (4) grower pigs. A total of four treatment groups (T1, T2, T3, and T4) were used in the experiment. T1 contained 0g Garlic and 0g Probiotics in a 50kg basal diet (Control). T2 had 50g of Probiotics in a 50kg basal diet. T3 contained 50g of Garlic in a 50kg basal diet. And T4 had 50g Garlic and 50g Probiotics in a 50kg basal diet.

A week before the arrival of the animals, the pens were cleaned, and subsequently disinfected to reduce the risk of disease infection. On arrival to the experimental pens, the pigs were dewormed and provided with a two-week period of equilibration to acclimatize to the environment, feed and water. The pigs were fed 4% of average body weight per pen at 0800h once every day and water was provided *ad-libitum*. Cleaning of the experimental pens was performed every morning.

3.5. The Test Materials

The treatments, designated and listed as follows, show the combination or mixture of the test materials.

Treatment 1. (T1) = Diet containing neither garlic nor probiotics, ie.control.

Treatment 2. (T2) =Diet containing no garlic but 50g probiotics per 50kg feed.

Treatment 3. (T3) = Diet containing 50g garlic but no probiotics per 50kg feed.

Treatment 4. (T4) = Diet containing 50g garlic and 50g probiotics per 50kg feed.

To ensure an even distribution of the probiotics and garlic materials in a given dietary treatment, 1kg of the basal diet was first isolated in each case and a required dose of the garlic or probiotics was then manually but thoroughly mixed with the basal diet to obtain a homogenous mixture which formed the treatments respectively.

3.6. MEASUREMENT OF PARAMETERS AND DATA COLLECTION

3.6.1. Linear body measurements

The initial body weight (IBWkg), height at whithers (HWcm), chest girth (CGcm), body flank (BFcm) and body length (BLcm) measurements were taken at the beginning of the experiment and subsequently bi -weekly till the end of the experiment. Each pig was trapped in a jute bag and hung on a spring balance with a maximum capacity of 100 kg, and the body weight measured. Other linear body measurements (cm) were obtained using a mathematical ruler and a canvass tape calibrated in centimeters.

3.6.2. Serum and hematological measurements

At the end of the experiment, a Veterinarian was invited from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka for investigation. One (1) pig was randomly selected from each replicate making a total of two (2) pigs per treatment. For the four (4) treatments under investigation, 8 blood samples were collected and used for the investigation. Each replicate had a separate syringe and needle to avoid contamination of blood samples between replicates and among treatments during blood collection which was done through the retro-bulbar plexus of the medial canthus of the eye of the pig. Blood samples were placed in micro tubes with Ethylene diamine tetra acetic acid (EDTA) as anti-coagulant for determining the haematological values (Ritchie *et al*., 1994).

3.6.3. Carcass evaluation

At the end of the experiment, 2 pigs were randomly selected from each treatment (1 pig from each replicate), slaughtered, and measurements of carcass parameters were taken.

3.7. Statistical model

$$X_{ij} = \mu + T_i + \beta_j + \epsilon_{ij}$$

Where

X_{ij}	=	any individual observation on the experimental unit
μ	=	overall mean
T_i	=	effects of i-th level of probiotics on the experimental animals
β_i	=	effects of j-th level of garlic on the experimental animals
ϵ_{ij}	=	random error associated with the observation.

3.8. Statistical Analysis

Data collected were subjected to a one-way Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) using a Statistical Software Package (SPSS, 2009). Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955) as contained in the statistical package.

CHAPTER FOUR

RESULTS AND DISCUSSION

Table 7: Performance characteristic of growing pigs fed varying dietary levels of Probiotics and Garlic

	TREATMENTS			
Parameters	T1 Control	T2 Probiotics	T3 Garlic	T4 Probiotics /Garlic
AIBW (kg)	16.88±1.09	16.88±0.13	16.75±0.14	16.50±1.44
AFBW (kg)	41.50±2.40^{b}	46.13±1.65^{a}	44.63±1.66^{a}	44.78±3.90^{a}
ADG (kg)	0.29±0.23	0.35±0.29	0.33±0.19	0.34±0.24
ADFI(kg)	1.44±0.66^{a}	1.26±0.69^{ab}	1.25±0.05^{ab}	1.16±0.07^{b}
FCR(kg)	4.97±0.39^{a}	3.60±0.23^{ab}	3.79±0.01^{ab}	3.41±0.24^{b}
Feed cost/kg gain (₦)	220.22±17.61^{a}	159.52±10.19^{b}	167.93±13.31^{b}	151.10±10.94^{b}

$_{a,\,b}$: mean values in a row with different superscripts are significantly different (P<0.05).

AIBW = Average initial body weight (kg/pig) AFBW = Average final body weight (kg/pig)BWG = Body weight gain (kg)ADG = Average daily gain(kg/pig)ADFI=Average daily feed intake(kg)FCR = Feed conversion ratio (feed/gain)Feed cost/kg gain .

Table 8 shows that the effects of including varying levels of Probiotics and Garlic on the performance of the pigs were significant (P<0.05. The AFBW of the pigs on T2, T3, and T4 were similar to each other (P>0.05) but higher (P<0.05) than the control (T1). The increase in the AFBW observed in this study can be attributed to the various modes of action of probiotics most especially: their ability to compete with pathogenic micro-organisms for nutrient and other growth factors in the gut, thereby making the rich source of nutrient available for absorption by the host animal for growth, their ability to break down carbohydrates and split them into their most basic elements and increase their absorption through the digestive system, their protein sparing effects whereby probiotics primarily use carbohydrates as growth medium while decreasing the pathogenic population in the gut and making more protein available for absorption by the host animal with a resultant increase in growth. The observed increase in body weight of pigs on probiotics is in agreement with ealier reports of Rolfe (2000) and Chang *et al* (2001). The similar (P>0.05) increase in AFBW in T3 can be ascribed to the ability of garlic (allicin) to inhibit the growth of pathogenic bacteria in the gut thereby resulting in increased nutrient absorption and a resultant increased body weight gain. Samanta and Dey (1991) also observed significant increase in body weight with piglets fed probiotics.

The lowest (P<0.05) mean values of AFBW observed in T1, and the similar (P>0.05) mean values of T2, T3 and T4 can be supported with the findings of (Chang *et al.,* 2001) who reported that probiotics has the ability to increase weight gain in farm animals when administered in adequate amount. It is also in agreement with the observation by (Oyetoye and Oyetoye, 2005) that probiotics improves the microbial ecosystem, nutrient synthesis and their bio-availability resulting in better growth performance in farm animals. In addition, it has also been reported to improve nutrient absorption in farm animals (Teeler and Vanabelle, 1991). However, there is a lot of inconsistency in the literature concerning the performance of pigs offered diets containing garlic. This inconsistency may be due to variable inclusion levels of garlic and in the allicin concentration of the garlic used (Grela *et al.,* 1998; Holden *et a*l., 2001; Holden and McKean, 2002; Corrigan *et al*., 2001). On the other hand the similarity of T4 (PG), T2 (P) and T3 (G) suggest neither synergism nor antagonism but rather no interaction.

The trend of result observed in AFBW is in agreement with the observation of (Gracia *et al.*, 2004) who reported that supplementation of pigs diet with piobiotics could benefit BWG during prestarter and the overall prestarter – finishing periods. The improvement in BWG can be attributed to the ability of probiotics to produce some useful enzymes (α – amylase, arabinase, cellulase, dextranase, levansucrase, maltase, alkaline protease, neutral protease and β – glucanase) that were found to improve feed efficiency and BWG (Chesson, 1994; Korneygay and Riseley, 1996).

Pigs on T1 had ADFI mean value of 1.44 ± 0.06 which differed ($P<0.05$) significantly from T4 (1.16 ± 0.07) but statistically similar ($P>0.05$) to ADFI mean values observed in T2 (1.26 ± 0.69), and T3 (1.25 ± 0.05). This result can be compared with the report by Chen *et al.* (2005) who found improved ADG, but reduced ADFI when grower pigs were placed on diets supplemented with L. *acidophilus*, *S. cervisae*, and *B. subtilis*.

Pigs on T1 (control) diet had a significantly ($P<0.05$) higher FCR value of (4.97 ± 0.39) that is similar to those in T2 (3.60 ± 0.23) and T3 (3.79 ± 0.01) but less than that of T4 (3.14 ± 0.24) (Table 8). This result can be compared also with the findings of Chang *et al.* (2001) where he observed that the inclusion of a mixture of different probiotics cultures improved ADG but not ADFI or FCR in growing pigs. The improved performance could be due to an improved digestibility on the supplemented diets as compared with the control.

Feed cost/kg gain of pigs fed T1 diets (220.22 ± 17.61) was higher ($P<0.05$) than those of T2 (159.52 ± 10.19), T3 (167.93 ± 13.31) and T4 (151.10 ± 10.94) that followed the same trend with the FCR. T2, T3 and T4 were statistically ($P>0.05$) similar while T4 had the lowest mean value (151.10 ± 10.94). The results show that feeding pigs on dietary combination of garlic and probiotics is cheaper but not the best production option for a farmer, since reduction in cost/kg gain is not only dependent on cheap feed but also on the production results obtained with this cheap feed when some feed additives were added (Ukachukwu and Anugwu, 1995).

Table 8: Linear body measurement of pigs fed varying dietary levels of Probiotics and Garlic

	Treatments			
Parameters	T1	T2	T3	T4
HW(cm)	47.54 ± 0.79^{bc}	53.00 ± 1.05^{a}	49.02 ± 0.77^{b}	46.17 ± 0.89^{c}
BF(cm)	52.50 ± 1.09^{ab}	55.75 ± 1.02^{a}	52.17 ± 0.99^{b}	53.29 ± 1.34^{ab}
BL(cm)	77.54 ± 1.40^{ab}	81.08 ± 1.54^{a}	78.96 ± 1.18^{ab}	75.02 ± 1.98^{b}
CG(cm)	67.46 ± 2.04^{b}	72.33 ± 1.28^{a}	69.58 ± 1.46^{ab}	65.02 ± 1.30^{b}

$_{a, b, c}$: mean values in a row with different superscripts are significantly different ($p<0.05$)

HW =Height at withers, BF=Body flank, BL=Body length, CG=Chest gait.

Results (Table 9) showed that height at withers (HW), body flank (BF), body length (BL) and chest gait circumference (CG) followed the same trend with the AFBW. The conformation of these characteristics with the AFBW (body growth) can be attributed to their varying nutrient utilization as well as genetic variations and the sex of the pigs used in this study. These parameters are indices of growth and grow as body parameters grow.

Table 9: Carcass and organ characteristics of growing pigs as affected by feeding levels of Probiotics and Garlic

	TREATMENTS			
Parameters	T1	T2	T3	T4
HW(g)	173.00±0.20^{c}	297.90±0.10^{a}	144.05±1.15^{d}	223.50±0.90^{b}
LW(g)	853.90±0.10^{b}	791.50±0.50^{c}	605.60±1.40^{d}	1024.30±5.10^{a}
KW(g)	107.50±0.80	130.85±0.35	67.90±0.30	86.40±69.80
PW(g)	59.25±0.25^{b}	72.50±0.50^{a}	38.45±0.55^{d}	55.35±1.15^{c}
HdW(kg)	4.34±0.00^{ab}	4.77±0.02^{a}	3.92±0.07^{b}	4.35±0.13^{ab}
HdW(g)	0.004±0.001^{ab}	0.005±0.001^{a}	0.003±0.001^{b}	0.004±0.001^{ab}
Carcass (kg)	30.00±0.00^{b}	34.75±0.25^{a}	23.00±1.50^{c}	31.50±1.00^{ab}
WL(g)	238.90±0.30^{c}	296.65±1.05^{b}	194.70±2.00^{d}	392.75±2.25^{a}
LP(cm)	21.90±0.10c	34.25±0.25^{a}	22.00±1.00^{c}	24.50±0.50^{b}
LSI (cm)	1301.00±1.00^{c}	1401.00±1.00^{a}	1047.00±10.00^{d}	2358.00±8.00^{b}
LLI(cm)	473.00±1.00^{b}	483.00±1.00^{a}	466.50±3.50^{b}	371.10±1.90^{c}

$_{a, b, c, d}$: mean values in a row with different superscripts are significantly different ($p<0.05$).

HW=Heart weight, LW=Liver weight, KW=Kidney weight, PW=Pancreas weight, HdW=Head weight, WL=Weight of lungs, LP=Length of pancreas, LSI=Length of small intestine, LLI=Length of large intestine.

Results showed that HW, KW, PW, HdW, carcass, LP and LLI followed the same trend with AFBW with T2 having the highest mean values (Table 10), except in carcass where T2 value is similar ($P>0.05$) to T4 value. The variation in the mean values observed on these parameters in T1, T3 and T4 suggested that supplementation of 1g/kg of probiotics in growing pigs' diet gave the best conformatory results with the AFBW. Hence, it did not have any deleterious effect on these parameters.

There were non significant ($P<0.05$) differences in (KW), (LSI) and (WL) among the treatment groups. Thus, these results conformed to the AFBW and can be attributed to non interaction effects of garlic and probiotics on the experimental animals. The enlargement of the liver with respect to this study can be attributed to the ability of the liver to detoxify any anti-nutritional factor that may have resulted from the digestion of garlic and probiotics by the animals (Madhusudhan, 1986). More so, the enlarged kidney observed can be ascribed to high deposition of uric acid related compounds (Opstevdt, 1988; Idowu and Eruvbetine, 2005). Increased levels of uric acid from excess purines (nitrogen-containing compounds) may accumulate in tissues, and form crystals. When the blood uric acid level rises above 7mg/dl, it becomes so deposited in the kidney such that kidney problems such as stones and gouts occur resulting in its enlargement.

Table 10: Serum biochemistry of growing pigs fed varying dietary levels of Garlic and Probiotics

	TREATMENTS			
Parameters	T1	T2	T3	T4
ALT (iu/l)	39.41±0.91	46.67±1.16	48.41±6.07	35.84±1.31 NS
AST (iu/l)	40.69±2.90	67.98±1.69	67.98±12.32	43.59±6.28 NS
ALP (iu/l)	53.70±5.75[b]	80.16±11.04[a]	68.80±1.69[ab]	58.14±3.06[ab]
BIL (mg/dl)	0.69±0.22[b]	1.57±0.11[a]	0.88±0.33[ab]	0.57±0.01[a]
TCRE (mg/dl)	1.89±0.22	1.69±0.02	1.53±0.06	1.83±0.70 NS
URE (mg/dl)	15.69±2.54	12.46±0.31	15.69±1.12	12.36±3.04 NS

a, b: mean values in a row with different superscripts are significantly different (P<0.05). NS;not significant. URE=Urea, ALT=Alanine amino transferase, AST=Asptate amino transferase, ALP=Alkaline phosphate, BIL=Bilirubin, TCRE=Total creatinine

Results (Table 11) revealed that there were no significant (P>0.05) differences in ALT, AST, TCRE, and URE values among the treatment groups. There were also non-significant (P>0.05) differences in ALP and BIL in T1 but similar (P>0.05) in T2, T3 and T4. The serum levels of ALT and AST observed in this study indicated that there were no cellular abnormalities among the experimental pigs offered the different treatments

Table 11: Serum Cholesterol values of growing pigs fed varying dietary levels of Probiotics and Garlic

	TREATMENTS			
Parameters	T1	T2	T3	T4
TCHL (mg/dl)	128.30±26.95[a]	87.33±9.16[c]	125.87±0.80[a]	106.20±12.94[b]
HDL (mg/dl)	33.88±1.53[a]	23.45±2.96[b]	33.96±3.77[a]	28.84±2.42[a]
TRIG (mg/dl)	115.21±35.50[a]	61.95±6.88[b]	111.95±16.30[a]	60.51±23.55[b]
VLDL (mg/dl)	23.04±7.10[a]	12.39±1.38[b]	22.39±3.26[a]	12.10±4.71[b]
LDL (mg/dl)	72.38±20.39[a]	51.49±10.75[b]	69.52±1.32[a]	65.25±10.65[a]

$_{a,\ b,\ c}$: mean values in a row with different superscripts are significantly different ($p<0.05$)

VLDL=Very low density lipoprotein, LDL=Low density lipoprotein, TRIG=Triglycerides, HDL=High density lipoprotein, TCHL=Total cholesterol.

The results (Table 12) from this study revealed that TCHL was similar (P>0.05) but highest (P<0.05) in T1 and T3 followed by T4 while T2 is the lowest. There were significant (P<0.05) differences in the HDL with T1, T3 and T4 having similar (P>0.05) mean values. TRIG and VLDL were also significantly (P<0.05) different with T1 and T3 being similar (P>0.05) but higher (P<0.05) than T2 and T4, which are themselves similar.

The low density lipoprotein (LDL) values were similar (P>0.05) in T1, T3 and T4 and these were higher (P<0.05) than T2 value. The lower mean value (51.49 ± 10.75) observed for LDL in T2 can be said to have given the best result in the reduction of serum cholesterol. This is because; LDL is the bad cholesterol in the meat that promotes health problems and cardiovascular disease. This result agreed with the observation of Ezema (2009) that supplementation with probiotics in layers diet significantly reduced egg cholesterol. This is achieved by the ability of probiotics to de-conjugate the bile acids. Since the extraction of de-conjugated bile acid is enhanced while cholesterol is its precursor, more molecules of cholesterol are spent for recovery of bile acids. As a result of the increased synthesis of these acids, the level of serum cholesterol becomes reduced.

These results are also supported by the report that elevated levels of HDL, VLDL and IDL (intermediate density lipoprotein) are thought to be antherogenic (National Heart, lung and blood institute, 2008). The high TCHL values observed in T1, T3 and T4 were as a result of their higher LDL (small dense particles) mean values (the bad cholesterol) while the low mean values for TCHL in T2 was due to low level in the LDL (mostly large particles) and smaller particles of HDL in which both the antheroma growth are usually low, or even negative for a given cholesterol concentration. Moreso, the results of TRIG was so because the low level of TCHL in T_2 will combine with the triglycerides and protein during lypolysis to give alternative energy source.

Table 12: Haematological values of growing pigs fed varying dietary levels of Garlic and Probiotics

	TREATMENTS				
Diff. Abs WBC, RBC & TWBC	T1	T2	T3	T4	
NEU	3.92±1.00b	5.66±0.15a	5.01±1.04a	7.49±1.07a	NS
LYM	7.94±0.49c	10.14±2.01b	10.44±0.86b	12.11±0.92a	NS
MON	0.00±0.00	0.08±2.01	0.09±0.09	0.10±0.10	NS
EUS	0.57±0.14	0.38±0.20	0.83±0.09	0.50±0.11	NS
BAS	0.00±0.00	0.08±0.08	0.00±0.00	0.00±0.00	NS
RBC	7.00±1.16	6.50±0.15	7.80±0.10	8.06±0.91	NS
TWBC	12.43 ± 1.63^{b}	16.33 ± 1.83^{ab}	16.35 ± 0.45^{ab}	20.20 ± 0.15^{a}	S
DIFF. WBC (%)					
NEU	31.00±4.00	35.00±3.00	30.50±5.50	37.00±5.00	NS
LYM	64.50±4.50	61.50±5.50	64.00±7.00	60.00±5.00	NS
MON	0.00±0.00	0.50±0.50	0.50±0.50	0.50±0.50	NS
EUS	4.50±0.50	2.50±1.50	5.00±1.00	2.50±0.50	NS
BAS	0.00±0.00	0.50±0.50	0.00±0.00	0.13±0.13	NS
PCV (%)	42.75±1.75	40.75±2.25	40.75±0.75	43.00±2.50	NS
HbC(g/dl)	12.59±0.38	12.78±0.95	12.02±0.19	12.40±0.85	NS

$_{a,\ b,\ c,\ d}$: mean values in a row with different superscripts are significantly different (p<0.05). NS;not significant. HbC= Haemoglobim count, PCV=Packed cell volume, BAS=Basophils, EUS=Eusinophils,MON=Monocytes,LYM=Lymphocites,NEU=Neutrophils,TWBC=Total whiteblood cells,RBC=Red blood cells,WBC=White blood cells,Abs=Absolute,DIFF=Differential

This study (Table 13) shows that the effects of the treatments on the PCV, RBC, and HbC are non-significant (P>0.05). The results on TWBC revealed that the effects of treatments was significantly (P<0.05) different with T_4 having similar (P>0.05) mean value as T2 and T3 but higher (P<0.05) than T1.

Considering the importance of the PCV as an indicator of blood dilution, the HbC as the measure of the ability of the animal to withstand some level of respiratory stress, the role of WBC in disease resistance with respect to generation of antiboidies and the process of phagocytosis, it could be reported that the dietary levels of probiotics and garlic in this study was not detrimental to their blood indices, especially the TWBC which was increased and fall within the normal range for pigs as indicated by Swenson and Reece (1993).

CHARPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Grower pigs exposed to supplementary feeding of garlic and probiotics responded favorably, especially when added in adequate amount. Therefore, feeding pigs on dietary additives of garlic or probiotics can be the best production option for farmers to achieve the best results when these feed supplements are added, in adequate amount, to their cheap and available feeds.

5.2. Recommendations

1. If probiotics should be used singly as feed additive for grower pigs, the dietary level of inclusion of 50g kg^{-1} of basal diet is beneficial and may improve production.
2. On other hand if only garlic should be used under similar condition, the dietary level of inclusion should not exceed 50g kg^{-1} of basal diet for optimum performance and economic gain.
3. Garlic and probiotics should not be used in combination as feed additives for grower pigs, since they showed neither synergitic nor antagonistic effects but rather suggests no interaction.

4. Given the recent concerns regarding the use of antibiotics as growth stimulating agent in animal production and their residual effects on the part of the consumers, which has demanded for alternative strategies to improve animal production without antibiotics, it is hereby recommended that the Federal Government of Nigeria should disband the use of antibiotics as animal feed additives while still making available and subsidizing the costs of probiotics and garlic to enable pig farmers purchase them at affordable and relatively cheap rates. Government should also, as a matter of urgency, be sensitive to these recommendations bearing in mind the residual effects of antibiotics on pork consumers to improve production and consumption.

REFERENCES

Abeke, F.O., Sekoni, A.A, Edwards, M., Kudu, M., Ubani, E.O and Out, M (2009). Effects of Naturally Occurring Egg Yolk Pigmenters on Performance, Egg Quality and Haematological Parameters of Shika Brown Layers. Trop. J, Anim.Sci. Dec.Vol.11 Pp 8-24.

Adetumbi M., G.T. Javor and B.H. Lan (1986). *Alluim Sativum* (garlic) inhibits lipid synthesis by Candide albicans. Antimicrobial agents are Chemotherapy 30: 495 – 501.

Adimorabi, M.B. Navidshad, J. Scifdavati and M. Royan (2006). Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. Jpn.Poult.Sci.43:278-282.

Aduku, A O (1992) Practical Livestock Feeds Production in the tropics. Feed Formulation; Retrieved online from.www.naeris.gov.ng/… on 23 Sept.2011.

Aengwanich, W. and Tanomtong, A. (2007). Blood cell characteristics and haematological values of free ranging – red jungle fowl (*Gallus gallus*) in Northern, Thailand. J. Bio. Sci., 7(4): 689 – 692.

Alakomi, H. L., E. Skytta, M. Saarela, T. Mattila-Sandholm, K. Latva-Kala and I.M. Halander. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl. Environ. Microbiol. 66: 2001 – 2005.

Alder, A.J and Holub, B.J. (1997). Effect of garlic and fish oil supplementation on serum lipid and lipoprotein concentration in hyper cholesterolemic men. American J. of Clinical Nutr., 65, 445 – 450.

Amagase, H. and J.A. Milner. (1993). Impact of various sources of garlic and their constituents on 7, 12 – dimethylbenzen [a] anthracene binding to mammary cell DNA. Carcinogenesis 14: 1627 – 1631.

American Society for Microbiology (2009). Biology Scholars research. Retrieved online from www.biologyscholars.org. on 14 April 2011.

Baird, D. (1977). Probiotics help boost feed efficiency. Feedstuffs 49: 11 – 12.

Bakhsh, R. and Khan R.V (1990). Influence of Onion and Chaunga on serum cholesterol, triglycerides, and total lipids in human subject. Sarhad. J. Agr. 6: 425 – 428.

Barber J, P.H, Brooks and J.L.Capenter. (1989) In: The Voluntary Food Intake of Pigs. Ed.Forbs JM, MA Varley and TLJ Lawrence. British Society of Anim.Producers. Occasional Publication No 13. Pp.103-104.

Bardia, A (1981). Effects of garlic on blood lipids in patients with coronary heart disease. A. M. J. china nutrition.34:2400-2403.

Bardia, A, S. K. Rthore, M.K. and N. Bku. (1975). The prospective actional essentials of onions and garlic in cholesterol fed rabbits. Atherosclerosis, 22:103-109.

Bentinck – Smith, J. (1969). Tex book of veterinary clinical Pathology. Waverly Press. USA.

Bhandari, S.K., Opapeju,F.O., and O.Krause (2010). Probiotics as a dietary additive for pigs. A review: In: The functions of probiotics within a Gastrointestinal Tract GIT. Retrieved online from www.medwelljournals.com.

Bjorkstén, B.E., Sepp, K. Julge, T. Voor and M. Mikelsaar (2001). Allergy development and the intestinal microflora during the first year of life. J. Allergy Clin. Immunol. 108: 516 – 520.

Block, E (1992). The Organ sulfur Chemistry of the genus *Allium* implicatios for the organic chemistry of sulfur. Angew. Chem. Int. Ed. 31:1135-1178.

Block, E. (1985). The organ sulfur chemistry of the genus *Allium* implications for the organic chemistry of sulfur. Angew. Chem. Int. Ed. 31: 1135 – 1178.

Block, E. (2010). Garlic and other Alliums: The Lore and the Science. Royal Society of Chemistry. ISBN 0854041907. Retrieved online from http://books.google.com./?id=6AB89RHV9ucC&printers=frontover. On 7-6-2010

Bomba ,A., Z.Jonecova, S. Gancacikova andNecova, R (2007). The potential benefits of probiotics in Animal production and health. Retrieved online from www.medwelljournals.com/fulltext. on 7-8-2010.

Breinholt, K.AL., Gowen, F.A and Nwosu, C.C. (1981). Influence of environmental and Animal factors on Day and Night Grazing Activity of Imported Holstein – Friesian Cows in the Humid Lowland Tropics of Nigeria. Trop. Anim. Prod. 6:4.

Brooks, P.H. and J.L. Carpenter. The water requirements of growing – finishing pigs: Theoretical and practical considerations. In: Recent Advances in Animal Nutrition. Ed. Haresign W. and DJA Cole. Butterworths; Boston. Pp 115 – 136. 1990.

Campbell, T.W. and Coles, E.H. (1986). Avian clinical pathology. In: Coles, E. (ed). Veterinary Clinical Pathology. Philadelphia, W.B. Saunders Coy. Pp 279 – 301.

Campbell,T.W.(1995).Avian haematology and cytology. Iowa, Iowa State University Press. Ames USA.

Chang , M.H.,Cheng,Y.C, and L.M Chen (1980) GARLIC (*Allium sativum*) SUPPLEMENTATION WITH…hypoglycemic effect of garlic. Retrieved online from www.pjps.pk/CD-PJPS-24/paper-24pd on 15 Sept 2010.

Chang, Y, Kim, W., Kim, Y, and Park, Y. (2001). Selection of a potential probiotic lactobacillus strain and subsequent in- vivo studies. Antonie Van Leeuwenbock Pp: 193 – 199.

Chen, Y.J., K. S. Son, B.J. Min, J.H. Cho, O.S. Kwon and I.H. Kim (2005). Effects of dietary probiotics on growth performance, nutrient digestibility, blood characteristics and fecal noxious gas content in growing pigs. Asian Aust. J. Anim. Sci. 18: 1464 – 1468.

Chen, Y.J., Min, B.J., Cho, J.H., Kwon, O.S., Son, K.S., Kim, H.J., Kim,. I.H., (2006). Effects of dietary – based probiotics on growth performance, nutrition digestibility, blood characteristics and fecal noxious gas content in finishing pigs. Asian – Aust. J. Anim. Sci. 19(4): 587 – 592.

Chesson, A. (1994). Probiotics and other intestinal mediators. In: Cole, D. J.A., Wiseman, J., Varley, M.A. (Eds.) Principles of Pig Science. Nottingham, University Press, Loughborough, U.K. pp. 197 – 214.

Coffin, L.D. (1957). Manual of Clinical Veterinary Pathology. Macmillan Publishers. Limited, London: P 138.

Corncionivoschi, N., A. Alemka., M. Clyne., F. Shanahan., T. Tompkins and B. Bourke (2010). Probiotic colonization of mucus – adherent: The effect of probiotics on Animal Health. Animal Science and Biotechnologies. 43 (1): 35.

Corrigan, B.P., Wolter, B.F., Elis, M. and Moreland, S. (2001). Effect of three dietary growth promoting additives on performance of nursery pigs. Journal of Animal Science 79 (Suppl. 1): 1885 (Abstract).

Corzo-Martinez M., N. Corzo and Mar Villanuel (2007). Biological Properties of onions and garlic. Trends in Food Science & Technology. 18: 609 – 625.

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

De Simone, C., Vesely,R and Bianchi, S.B (1993) The role of probiotics in modulation of the Immune system in man and animals. Int. J. Immunother 9: 23 – 28.4.

Duncan, D.E. (1955). New Multiple Range Test. Biometrics 11: 1 – 42.

Dunne, C., O'Mahony, L, Murphy, L., O'Halloran, S., Feeney, M, Flynn S, Fitzgerald G, Daly C, Kiely B, O'Sullivan G, Shanahan F, Collins JK (1999). Probiotics; from myth to reality – Demonstration of functionality in animal models of Disease and human clinical trails. Antonie Van Leeuwenhoek 76: 279 – 292.

Energy Research Centre, UNN (2008). Annual Weather Record of University of Nigeria, Nsukka.

Ensminger, M.E., Oldfield and Heinemann, W.N. (1996). Feeds and Nutrition. The Ensminger Publishing Coyi Clovis California, USA. Pp 324 – 366.

Ezema, C., (2007). The performance of broilers fed palm kernel cake – based diet supplemented with bioactive yeast. M.Sc. Thesis. Dept. of Animal Health and Production, University of Nigeria, Nsukka.

FAO (2005) GUIDELINE S FOR EVALUATION OF PROBIOTICS IN FOOD. Food and Agricultural Organization. Goog Clinical Practices (GCP) J. Farm Pract. 54 (7): 613-20.

FAO/WHO (2005). Health and Nutritional Properties of probiotics in food including Powdered Milk with Live Lactic Acid Bacteria. Report of joint FAO/WHO experts. Consultant on evaluation of health and nutritional properties of probiotics in food including powdered milk with live lactic acid bacteria. From wikipedia, the free encyclopedia.

Frandson, R.D. (1986). Endocrinology. In Anatomy and Physiology of farm animals. Philadelphia, Lea and Febiger Pp 233 – 247, 481 - 507.

Franscico, G., Gabriela, P., Gerard,C and Seppo, S (1995). Probiotics as a dietary additive for pigs. A review. Retrieved online from www.medwelljournals.com. On 19-9-2011.

Fraser, D., J. F Patience, P A Philips and J M Mcleese (1990). Water for pigs and lactating sows. Quantity,quality and quandaries. In. Recent Advances in Animal Nutrition. Ed. Haresign W and D J A Cole. Butterworths; Bosten. Pp. 137-160.

Freter, R. (1992). Factors affecting the microbiology of the gut. In: Fuller, R. (Ed). probiotics the scientific Basis. Chapman & Hall London, Pp. 111 – 114.

Fuller, R. (1989). "Probiotics in man and animals". The Journal of Applied Bacteriology 66 (5): 365 – 378.

Gernot, K. (2005). Spice pages'. Garlic (*Allium sativum*). Retrieved from Retrieved on line from http://www.uni-graz.at/~katzer/engl./Alli_sat.html on 2007 - 08 – 28.

Giang, H. H, T.Q. Viet, B. Ogle and J.E Lindberg (2010). Growth performance, digestibility, gut environment and health status in weaned piglets fed diet supplemented with potentially probiotics complexes of lactic acid bacteria. Livest. Sci. 129:95-103.

Gracia, M.I., Hanson, S., Sanchez, J., Medel, P., Imasde Agropecuaria, S.L., (2004). Efficacy of addition of B. Licheniformis and B. substilis in pig diets from weaning to slaughter. J. Anim. Sci. 82 (Suppl. 1), 26.

Grela, E.R., Krusinski, R. and Matras, J. (1998). Efficacy of diets with antibiotic and herb mixture additives in feeding of growing – finishing pigs. Journal of Animal and Feed Science 7: 171 – 175.

Grupta, P.P. (1973). Normal haematological and biochemical parameters of Indian pigs (*Sus scrofa domestica*). Indian Vet. J. 50: 406 – 409.

Han, J., L. Lawson, G. Han and P. Han. (1995). A spectrophotometeric method for quantitative determination on *allium* and total garlic thiosulfnate. Anal. Biochem., 225, 157 – 160.

Hatakka, K., Nase, L and Savilahti (2002). Effect of long term consumption of probiotiics bacterium in children attending day care centre. Int. J. Imunother 9: 92-96.

Hawkey, C., Hart, M.J. and Samour, H.J. (1994). Age related haematological changes in chicken flamingo (*Phoenicopterus chiliensis*). Avian Path., 13 (2): 223 – 229.

Hentges, D.J. (1992). Gut flora in disease resistance. In: Fuller, R. (ed). Probiotics the scientific Basis. Chapman and Hall, London, Pp 87 – 11.

Holden, P.J and McKean, J. D (2002). Botanicals for nursery pigs. Journal of Animal Science 80(1):41.

Holden, P.J., McKean, J.D. and Franzeburg, E. (2001). Botanicals as part of an intergrated value – added pork production system. Leopold Centre Progress report, Volume 10, Iowa State, University, USA.

Holness, D.H. (2005). Pigs. The Tropical Agriculturist, CTA. (The Technical Centre for Agriculture and Rural Co-operation). Editors: Rene Coste and Anthony J. Smith. Revised Ed. Macmillam Publishers Ltd, Oxford. PP 152.

Hong, H.A., Babosa, T.M., Duc, L.H and Henriques, A.O (2002) Effects of dietary Bacillus –based Probiotics on Growth AJAS. Retrieved online from www.ajas.info/editor/.../19-25.pdf on 25-9-2011.

Hong, H.A., L.H. Duc and S.M. Cutting. (2005). The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev. 29: 813 – 835.

Idowu, O.M.O. and Eruvbetine, D. (2005). Performance, digestibility and carcass yield of broiler chicken fed diets containing two types of fish meal. Nig. J. Anim. Prod. 32 (2): 204 – 214.

Jain, N.C. (1993). Essentials of veterinary haematology. Philadelphia, Lea and Febiger USA. Pp. 133 – 168

Jeffrey, J.S (1999). Probiotics as a Dietary Additive for pigs. Texas Journal of Science 51(2): 195-198.

Jessek S, Kalinowska R, Knelch D, Pawiak R (1992). Effect of Biogen probiotic addition on reproduction results and physiological indices in pigs. 2. Effect of Biogen –T dietary additive on results of rearing weaners. Rocz Nauk Zootec 1992;31:229

John, C. Rea and Trygve, L (2011). Review of vitamin requirements of swine. Department of Animal Science, University of Missouri.

Johnson, E., Conway, P., Fuller, R., (1992). Probiotics for pigs. Probiotics the scientific Basis. Chapman & Hall, London; Pp 259 – 316.

Kero,J., M. Gisssler, M.M. Gronlund, P. Kero, P. Koskinem, E. Hemminiki and E.. Isolauri (2002). Mode of delivery and asthma- is there a connection? Pediatr. Res.52:6-11.

Kornegay, E. T. and Riseley, C.R., (1996). Nutrient Digestibility of a cornsoyabean meal diets as influenced by Bacillus products fed to finishing swine. J. Anim. Sci. 74: 799 – 805.

Kyriakis, A. S.C (2004). Field evaluation of the efficacy of a prbiotic containing *B. Lichinformis* and *B. subtilis* spores, on the health status and performance of sows and their litters. J. Anim. Physiol. Anim. Nutr. 88, 381 – 392.

Kyriakis,C.S., Alexopoolos, C, Georgoulakis,I. E, Tzivara, A, and Govaris, A (2004). Field evaluation of the effect of a probiotc containing *Bacillus licheniformis* and *Bacillus subtilis* on the health status, performance and carcass quality of grower and finisher pigs. Retrieved on line from www.ncbi.nlm.nih.gov/pubmed/15485567. Aug51(6): 302-12.

Kyriazakis, Llias and Colin T. W (2006). Whittemore's science and practice of pig production 3rd Edition. Black well publishing Ames, IA.

Laswai, H. C; Givens, R; Fuller, M.F and Cornwell, G.L. (1997). Studies on *in situ* degradation of feeds in the rumen II. Effect of species, bag motility and incubation sequence on dry matter disappearance. Animal Feed science and Technology 65: 222 – 226.

Lewis, Austin, J. and L. Lee Southern (2001). Swine Nutrition. 2nd Edition. CRC Press. Bocca Ration Florida. PP 381 – 400.

Luley, C., Lehmann-Leo, W.,Mcjjller, B and Martin, T (1986). Lack of efficacy of dried garlic in patients with hyperlipoprotein. Retrieved online from www.ncbi.nlm.nch.gov/pubmed/3521619 on 4-6-2012.

Madhusudhan, K; Ranesh, H.P., Ogawa, T., Sasaoka, K. and Singh, N. (1986). Detoxification of commercial linseed meal for use in broiler ration. Poult. Sci. 65: 164 – 171.

Madson, K.L., (2001). The use of probiotics in gastrointestinal disease. Can. J. Gastroenterol. 15: 817 – 822.

Malago, J.J. and Koninkx, J.F.T.G (2011). Probiotic Bacteria and Enteric Ifections.Cytoprotection by Probiotic Bacteria. Retrieved online from www.springer.com/.../978-94-007-0385 on 12-4-2011.

McGee, Harold (2004). McGee on food and cooking. Hodder and Stoughton. P. 70. ISBN 0340831499.

Meng, C.L. and Shyu, K.W. (1990). Inhibition of experimental cacinogenesis by painting with garlic extract. Nutr. Cancer. 14; 207 – 217.

Miller, E.R., Ullery, D.E; Ackerman, I.M; Schmidt, D.A; Luecke, R.W. and J.A. Hoefer (1961). Swine haematology from birth to maturity I. Serum protein II Erythrocyte Population and size. J of Anim. Sci. 20: 31 – 89.

National Cholesterol Education Program Report (1988). Expert Parnel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. "The Expert Panel" Arch. Intern. Med 148(1):36-69. doi: 10/001.

National Heart, Lung and Blood Institute (2008). Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. (Adult Treatment Panel 111) Final Report. Retrieved on line from www.nhibi.nih.gov/guidlines/cholesterol/atp3full.pdf. 10-27.

Nienaber,J. A and G. L. Halu (1984). Effects of water flow restriction and environmental factors on performance of nursery pigs. J. Anim. Sci. 59:1423-1479.

Ofomata, G. E. K. (1975). Soil erosion. In Nigeria in Maps. Eastern States. Benin, Ethiope Pub. House. Pp43-45

Opstevdt, J. (1988). Influence of drying and smoking on protein quality. In: Fish smoking and drying. Burt, J.R. (Ed.). Elsevier Applied Sci. Ltd., USA. Pp 23 – 36.

Oyetoye, V.O. and Oyetoye, F.L. (2005). Potential of probiotics as biotherapeutic agents targeting the innate immune system. Afr. J. Biotech. 4: 123 – 127.

Paul Bergner (1996).The Healing Power of garlic. Primer Publishing. ISBN-7615-0098-7. Retrieved online from www.livestrong.com/.../191642 on 2-8-2011.

Pena, N; Auro, H., and Sumano H. (1988) A Comparative trial of garlic,its extracts and ammonia titrate as anthelmintic in cap. J. Ethnopacol 1988 Dec; 24(2-3):199-203.

Qureshi, A.A., N. Abuirmeileh, Z.Z. Din, C.E. Elson, and W.C. Burger (1983). Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. Lipids 18: 343 – 348.

Ramakrishma. R.R; Platel K. and K. Srinivasan (2003). In vitro influence of spices and spice – active principleson digestive enzymes of rat pancreas and small intestine. Nahrung 47:408-412.

Ramirez, C.G., Miller, E.R; Ullery, D.E. and J.A. Hoefer (1963). Swine haematology from birth to maturity, III. Blood volume of the nursing pig. J. of Anim. Sci. 22: 1068 – 1077.

Ress, L.P., S.F. Minney, N.J. Plummer, J.H. Slatter and D.A. Skyrme, (1993). A quantitative assessment of the antinucrobial activity of garlic (*Allium sativum*). World Journal of Microbiology and Biotehcnology 9: 303-307.

Rolfe, R.D (2000). The role of probiotic in the control of gastrointestinal health. J. Nutr., 130: 396S – 402S.

Rothenburg, R.E. (1976). The New Illustrated Medical Encyclopedia (4th ed.). Abradate Press Publishers, New York, Pp: 384 – 386.

Samanta, A.R. and A. Dey, (1991). Effect of feeding garlic (*Allium sativium Linn*.) as a growth promoter in Japanses quails (*Coturnix Coturmix Japonica*) and its influence on dressing parameters. Indian J. Poult. Sc. 26: 142 – 145.

Savage,D.C (1969). Probiotics as a Dietary Additive for pigs: A Review… J. of Anim and Vet,Advances 10(16):2127-2134.

Schalm, O.W., Jain, N.C. and Caroll, E.J. (1975). Veterinary Hematology. 3rd edn. Lea and Febiger. Philadelphia, USA. Pp 471 – 538.

Schrezenemier and De Vrese (2001). Viability of *Bifidobacteria* strains in yogurt with added oat Beta-glucan and corn starch during cold storage.

Serres, H. (1992). Manual of Pig Production in the Tropics. 2nd ed. AB International Publishers. Pp 57:491-498

Servin, A.L. (2004). Antagonistic activities of *Lactobacilli* and *bifidobacteria* against microbial pathogens. FEMS Microbiology Reviews 28: 1 – 5.

Sharma, R.K., R.A. Singh, R.N. Pal, and C.K. Aggarwal (1979). Cholesterol contents of chicken eggs as affected by feeding garlic, sarpagandha and nicotinic acid. Haryana Agric. Univ. J. Res., 9: 263 – 265.

Sklan, D., Y. N. Burner and H.D. Rabinowitch. (1992). The effects of dietary onion and garlic on hepatic lipid concentration and activity of antioxidative enzymes in chicks. J. Nutr. Biochem. 3: 322 – 325.

Smith,A.J.(2001).Poultry.The tropical Agriculturist. Revised ed.Macmillan Education Ltd.London.Pp 1-11.

Sohary Daniel and Hopt Maria (2000). Domestication of plants in the old world, 3rd Ed. Oxford University Press, P: 197.

Sonaiya, E.B., Williams, A.R. and Oni, S.A. (1986). A Biologic and Economic Appraisal of Broiler Production up to 16 weeks. J. Anim. Sci. Res. 6(2): 115 – 125.

Sovová M. and Sova P. (2004). Pharmaceutical importance of *Allium sativium* L. 5. Hypolipidemic effects in vitro and in vivo (in Czech). Ceska Slov Farm 53 (3): 117 – 23 PMID 15218732.

SPSS (2009). Discovering statistics using SPSS google books. Retrieved on line from books.google.com/…/ discorvering statistics. SAGE PUBLICATIONS. Jan.23

Starvric,S and Kornegay, E.T (1995). Lactic Acid bacteria: Microbiological and functional aspects. Retrieved on line from www.igitur_archive.libery.ua.nl. On 20-10-2011.

Swenson, M.J. and Reece, W.O. (1993). Dukes Physiology of Domestic Animals. 11th ed. Cornel University Press. New York Pp. 22 – 48, 962.

Takahashi, Y., Nishimura, H., Meada, M and Kondo, M (1998). Effects of probiotics – vitacogen and β1-4 Galactv-oligosaccharides. Retrieved online from www.ajas.info/Editor/manuscript/upload/17_55.pdf. on 4-3-2011.

Tannock, G.W. (2003). Probiotics: time for a dose realism. Current issues in intestinal Microbiology 4 (2): 33 – 42. PMID 14503687.

Taras (2004). Performance, diarrhea incidence, and occurrence of Echerichia coli. Jas.fass.org./content/84/3/608.ful.pdf.

Teeler, E. and Vanabelle, M. (1991). Probiotics: Facts and Fiction, Mededelingen van defacultait landbouwwetenschappen, Rijksumversitet Gent., 56: 1591 – 1599.

Trafalska, E. and Grzybowska, K. (2004). Probiotics an alternative for antibiotics. Wiad Lek. Pg. 57: 491 – 498.

Ukachukwu, S.N. and Anugwa, F.O.I. (1995). Bio-economic of feeding raw or heat treated soyabeans to broilers. Nig. J. of Anim. Production 22(2): 137 – 140.

Upadhyay, A (1980) Effect of alicine on various fungal pathogens. Retrieved online from www.parul.acim/files/Research_publi...35: 109-119. 4-3-2011.

USDA, National Nutrient Database for Standard References 2008 Release 21" (PDF). United States Department of Agriculture. Retrieved 10 – 24.

Vaiman, M.; Dubiez, R.; Colson, K.; and P. Nizza (1968). Haematolgoical data on the pig for use in radiobiology. Rev. Med. Vet 119: 129 (Abst).

Vanabelle, M. (2001). Current status and future perspectives in E.U. for antibiotics, probiotics, enzymes and organic acids in animal nutrition. In: Gut Environment of pigs. (Ed. A. Piva, K.E. Bach Knudsen and J.E Lindberg). Nottingham University Press. Pp. 231 – 256.

Wang, J.P. J.H. Lee, H.J. Kim, L. Yan, S.M. Houg; and I.H. Kim (2010). Journal of Anim. Sci. 88 (e – suppl. 2): 101.

Wang, Y., J.H. Cho., Y.J. Chen, J.S. Yoo., Y. Huang, H.J. Kim and I.H. Kim (2009). The effect of probiotic BioPlus 2B ® on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. Livest. Sci. 120: 35 – 42.

Wargovich, M.J., Woods, C., Eng. V.W.S., Stephen, C. and Gray, K. (1988). Chemo prevention of N – nitrosome thylbenzylamine – induced esophageal cancer in rats by the naturally occurng diallyl Sulphide. Cancer Res. 48, 6872 – 6875.

Warshafsky, S., Kamer R.S. and Sivak L. (1993). Effect of garlic on total serum cholesterol. A meta – analysis. Annals of Internal Medicine, 119: 599 – 605.

Weichselbaum, E. (2009). Probiotics and health: a review of the evidence. Nutrition Bulletin 34: 340 – 373.

Wikipedia (2009). Mean corpuscular hemoglobin concentration. Retrieved online from http://en.wikipedia.org/wiki/mean_corpuscular_hemoglobin_concentration on 05-03-2011.

Woodliff, H.J. and R. P. Hermann (1978). Concise Haematology. 2nd Edition. Edward Arnold Publishers Limited, London.

Yan, L., Q.W. Meng, X. Ao, T.X. Zhou, J.S. Yoo, H.J. Kim and I.H. Kim (2011). Livestock Science, 137: 255 – 259.

Yeh, Y.Y. and Liu, L. (2001). Cholesterol lowering effects on garlic extracts and organosulfier compounds: human and animal studies. J. Nutrition 131:9895-9935.

Yoruk, (2004). The effects of supplementation of huniates and probiotics on egg production and quality parameter during the late laying period in hens. Poult. Sci. Jan., 83(1): 84 – 86.

Yu, P., J.T. Huber, C.B. Theurer, K.C. Chen and Nussio, L.G (2008). Probiotics as a dietary additive for pigs. Retrieved online from www.medwelljounals.com. 10-7-2011.