Effect of nanozinc oxide supplement on growth performance characteristics, physiological parameters and immune response of weaner Large White piglets

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Dissertation accepted in fulfilment of the requirements for the degree Masters in Animal Science at the North West University

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Graduation ceremony: October 2022

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Declaration

I, Bontle Mokone, student number: 22477772, Department of Animal Science, North West University (Mahikeng Campus) hereby submit this thesis in fulfilment of the degree of Masters in Animal Sciences. I declare that the entirety of the work contained herein is my original work and that I have not previously, in its entirety or part submitted it for any qualification. The work has been done under the supervision of Dr. L.E Motsei and Dr. A.O Yusuf.

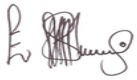
Signed Date

…… ……

Bontle Mokone

This dissertation has been submitted with my approval as a university supervisor and would certify that the requirements for the applicable Master's degree rules and regulations have been fulfilled.

Signature and Date Signature and Date



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Dr. L.E Motsei Dr. A.O Yusuf

Acknowledgment

First and foremost, praises and thanks to the Almighty God for his showers of blessings throughout my research work and for the strength to complete it successfully.

I would like to express my sincere gratitude in all humbleness to my supervisors, Dr. L.E Motsei and Dr. A.O Yusuf for their invaluable guidance throughout this research. Their sincerity, empathy, motivation, understanding, and support coupled with their constructive criticism which makes this research a success.

I am extremely grateful to my parents; Mr. and Mrs. Mokoena for their care, love, support, and prayers all through, and sacrifices for educating me and preparing me for the future are appreciated. I am very much grateful to my husband, Dr. Enioluwa Ijatuyi for his love, support, patience, encouragement, and help during this whole project, my sons, Jonathan, Caleb and Uel for their patience and understanding and encouragement through this phase of my life. My loving sister, Palesa for her prayers, support, and help during my data collection and always being there for me and her help with my children during my busy schedule. My brother Seitshiro for taking this research as if it were his own, for the sacrifices and the time he dedicated to this research (the feeding, cleaning, blood collection, weighing, and even the data collection), I am deeply humbled and indebted to you for everything. My mother-in-law, Dr. Mrs. Abiolu, and father-in-law Late Mr. Abiolu for their endless prayers, motivation, encouragement, and support. My brothers, Mr. Abels, Mr. Philip Abiolu, Dr. Patrick, and sisters in law, Dr. Rhoda Patrick and Mrs. Abels for their love, prayers, help, and support. Ous Nima and Bro Buti for all the administrative support. Dr. Makhosonke, Dr. Ntumba, Mr. Mogapi, Mr. Ijane, and their students who helped me with the collection of blood. The Animal Science diploma students, Thato,

Nketse, Tumelo, Justice, Sasa, Bokamoso for always being there when I needed help. Mr. and Mrs. Mokgatlhe, Lala, Leruo, and Patrick for always being there and availing the necessary resources for me when I needed them. And last but not least, Mr. and Mrs. Velile for allowing me to use their farm for my experiment and also their support and motivation. I am very grateful to everyone and it is such an honour to have worked with you all. Any attempt at any level would not be successful without all of you as part of this work. I am very much grateful, and God bless you all.

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List of Abbreviations

AMA- American Medical Association

ALT- Alanine aminotransferase

APHA- American Public Health Association

AST- Aspartate aminotransferase

CIWF – Compassion in World Farming

DAF – Department of Agriculture and Fisheries

GPAs- Growth Promoting Antibiotics

Kg – Kilogram

N.d – No date

Nm- Nanometers

NLR- Nod-like Receptors

NRC – National Research Council

nZnO – Nano zinc oxide

RSA – Republic of South Africa

TLR- Toll-like Receptor

WHO- World Health Organization

Zn – Zinc

RI – Reference intervals

Abstract

This study was conducted to evaluate the effects of dietary Nano zinc oxide on growth performance, physiological parameters, and immune response of weaner piglets. Thirty (30) piglets with an average weight of 7.6 $\pm$ 0.54 kg were reared intensively in a local farm. Piglets of mixed genders were randomly allotted to 5 different treatments: 200mg/kg, 400mg/kg, 600mg/kg of Nano zinc oxide, positive control (Tylosin 10%) and the negative control (no additive) supplemented via the feed in a completely randomized design. The results showed the treatments of Nano zinc oxide had a significant ($P<0.05$) effect on the body weight gain and feed conversion ratio. The piglets supplemented with 200mg/kg of Nano zinc oxide had a significant ($P<0.05$) final body weight gain while those supplemented with 400mg/kg and 600mg/kg of Nano zinc oxide had higher comparable weight gains and the control groups had the least weights. Pigs supplemented with Nano zinc oxide in all the levels had significant ($P<0.05$) comparable values on slaughter weights while the positive control had the least significant ($P>0.05$) performance in back fat thickness and meat pH after 24 hours of slaughter. Pigs supplemented with 200mg/kg and 400mg/kg of Nano zinc oxide gave a darker meat colour while the other meat quality parameters were not significantly ($P>0.05$) affected by the treatments. The albumin, total protein, phosphate ions, alpha globulin, and beta globulin were significantly ($P<0.05$) influenced by the treatments of Nano zinc oxide. Creatinine, aspartate aminotransferase, alanine aminotransferase, calcium ion, and Fe were similar ($P>0.05$) across the treatment groups. Pigs supplemented with 600mg/kg of Nano zinc had the highest albumin concentrations with the least values observed in 200mg/kg and 400mg/kg of Nano zinc oxide groups. Pigs offered Tylosin 10% and 600mg/kg of Nano zinc oxide had higher comparable total protein, while those fed with the diets with no additives had the lowest total protein

concentration. Higher similar phosphorus ions were recorded in Tylosin 10% and Nano zinc oxide supplemented groups compared to the negative control group. A similar trend was observed for alpha and beta globulin. The treatments had a significance ($P<0.05$) influence on the size of the villi. The supplementation of 600mg/kg of Nano zinc oxide had the greatest values of height while the groups supplemented with 200mg/kg and 400mg/kg of Nano zinc oxide had a similar trend and the control had the least comparable values of villi height. It could be concluded that the supplementation of Nano zinc oxide at a dietary dose of 600mg/kg gave the best performance in terms of intestinal morphology (villus height), growth performance, meat quality, and immune response.

CHAPTER 1

INTRODUCTION

1.0 Background

In animal nutrition, it is a satisfactory option to use Nanominerals because they are inexpensive, and they take less time to be effective. They have proven to have a more positive effect as growth enhancers, immune modulators, and antibiotics than conventional counterparts. They promote reproduction and alter the pattern of rumen fermentation in animal feeds when supplemented. The Nano mineral particle size affects functional processes such as chemical, catalytic, and biological effects (Swain *et al*., 2015).

Piglets offered diets with added amount of zinc oxide excrete between 60 and 80% of the consumed volume; i.e. the greater amount of zinc oxide ingested by the piglets, the greater the amount that will be found in the excreta (Carlson *et al*., 2004). Nanoparticles of zinc aid in the decrease of the amount of zinc excreted in animal feaces and consequently the environment (Barreto & Conte-Junior, 2018).

Sindhura *et al*. (2014) highlighted that the application of Nano materials in agriculture and animal husbandry is of utmost importance. Stoimenov *et al*. (2002) reported that Nano minerals have a particle size of 1-100 nm and are very resilient under high temperature and pressure and that they can be readily absorbed by the gastrointestinal tract. Zaboli *et al*. (2013) emphasized that because of the greater surface area, Nano minerals result in stronger interaction with other organic and inorganic substances.

Nano mineral feeding in livestock has, according to Swain *et al*. (2015), developed positive feed reactions as an alternative to traditional mineral sources. Feeding the animals with Zinc has provided promising results in health, immunity, and reproduction. Swain *et al*. (2015), Yang and Sun (2006), Mishra *et al*. (2014), and Lina *et al*. (2009) in their studies have found that Nano zinc oxide enhances growth, improves weaner piglets and poultry feed quality. Yang and Sun (2006) observed a substantial reduction in the incidences of diarrhoea if accompanied by Nano zinc oxide-rated doses in the feed fed to piglets in a study performed on early-weaned piglets. Hill and Li (2017) also indicated that the possibility of using Nanoparticles to enhance meat and egg quality has also been investigated.

Tsai *et al*. (2016) stated that Zinc is an essential trace element required for the structure and maintenance of the skeleton and act as cofactor in many metabolic processes that are necessary for hormone secretion. Prasad (1991), Suchy, *et al*. (1998) and Parashuramulu, *et al*. (2015) all identified that DNA synthesis, gene expression and cellular division, vision photochemistry all enhance the immune system through creating energy, protein synthesis and cell membranes. Zinc is an essential mineral for humoral and cell mediated immune responses (Gruber & Rink, 2013) and plays an important role in antioxidant defence system (Bray & Bettger, 1990). Weaning in commercial pig farms is an abrupt process that commonly takes place between the ages of 3-4 weeks, where piglets are exposed to different simultaneous stressors. These abrupt changes are often accompanied by a transient low feed intake, poor growth, intestinal symbiosis and diarrhoea post weaning thus compromising animal health and welfare, increasing piglet mortality and causing economic loss (Seve, 2000; Bruininx, *et al*., 2002; Heo, *et al*., 2013; Gresse, *et al*., 2017; Everaert, *et al*., 2017; Kim, *et al*., 2017). At the time of weaning, the GIT of a young piglet is still developing and undergoing rapid changes in the gut microbiota colonisation (Kim, *et al*.,

2017), digestive system and immune development (Pajarillo, *et al*., 2014, Frese, *et al*., 2015). Importantly, the gut micro-biome has been recognized to play a vital role in overall animal health and development especially in early life (Merrifield, *et al*., 2016, Schokker, *et al*., 2014).

Antibiotics growth promoters (AGPs; like Tylosin) are substances that kill or inhibit bacteria and are administered in sub therapeutic doses to improve animal growth (Boamah, *et al*., 2016; Dewey *et al*., 1999). Antimicrobials have been used in animal agriculture for more than 60 years and have contributed to the improvement of livestock health and productivity (Choudhury, *et al*., 2021). Antimicrobials fed to livestock at subtherapeutic levels promote growth by improving feed efficiency (Choudhury, *et al*., 2021). These AGPs are considered as the most effective way to maintain and improve feed efficiency (Elliot, *et al*., 2017) as their use in livestock diets have increased the farmers and agricultural output thereby reducing the negative impacts of stress in livestock such as post weaning diarrhoea in pigs (Boamah, *et al*., 2016).

The mechanism of growth promotion is not quite known but probably includes alterations in the compositions of the intestinal microbiome of animals (Kim, *et al*., 2014; Kim, *et al*., 2017). The long duration of antimicrobial use at sub therapeutic concentrations contributes to the selection and maintenance of antimicrobial resistant bacteria (Seve, 2000). Antimicrobial growth promoters are thought to have additional beneficial effects on animals such as better health, reduced morbidity and mortality rates (Choudhury, *et al*., 2021), reduced risk of subclinical infectious disease, and reduced risk of carrying zoonotic pathogens. All of these contribute to the production of animals that are healthier and more uniform in size at the time of slaughter (Casewell, *et al*., 2003), which may lead to decreased processing errors in slaughter plants and reduced carcass contamination, thereby improving human health (Hurd, *et al*., 2008; Hurd**,** *et al*., 2012; Singer, *et al*., 2007) .

Tylosin has the antimicrobial activities against most gram positive *bacterias mycoplasma* spp and a limited number of gram negative bacteria (Giguere, *et al*., 2013). Although tylosin does not have direct antimicrobial activity against *Samonella-enterica*, tylosin reduces the duration of shedding in pigs experimentally challenged with *Samonella-enterica serova Typhimurium* (Shryock, *et al*., 1998).

In pig production, tylosin is used therapeutically for several diseases including atrophic rhinitis and proliferative enteropathy (Giguere, *et al.*, 2013). *Samonella-enterica* is one of the most important bacterial food borne pathogens and is highly prevalent in pig herds. Pork products are known to be a source of this pathogen (Kim, *et al*., 2014). However, the sub therapeutic use of antibiotics as growth supporters results in the development and subsequent transmission of resistant bacteria between animals and from animals to humans (Van Der Fels-Klerx, *et al.*, 2011). Various studies have shown that AGPs use affects the intestinal bacterial of animals and leads to bacterial evolution and, changes in bacterial survival and bacterial gene mutations leading to antibiotic resistance development (Lee, *et al*., 2017). As a result, the EU has restricted the use of AGPs since 1999 and completely banned their use in 2006 (Burow, *et al*., 2014).

1.1 Problem statement

Graham *et al*. (2007) indicated that the use of Growth Promoting Antibiotics (GPAs) in animals raised for food is associated with a considerable controversy with the use of human antibiotics to promote growth in animals raised for food. The use of antibiotics as growth promoters in animal feed has raised concerns about the increased resistance by pathogens to antibiotics and the existence of antibiotic residues in animal products that end up consumed by humans (Ronquillo & Hernandez, 2017). The World Health Organization, the American Medical Association, and

the American Public Health Association have all advocated for a ban on GPAs, claiming that their use results in an uptick in antibiotic-resistant infections in humans. There are very few studies that have provided evidence on the significant effect of GPAs on the growth rate, feed conversion efficiency, and the production qualities of the farm animals (Wang, *et al.*, 2017). There is a gap in exploring the novel alternatives to antibiotic feed additives to enhance animal performance and also to improve immune response and therefore reduce the antibiotic resistant infections by pathogens.

1.2 Justification

Nano zinc oxide acts as an antibacterial agent and also a growth promoting agent. It alters immunity and animal reproduction constraints and also prevents environmental contamination. The food industry uses zinc as a feed particle and also in food packaging because of its antimicrobial properties (Swain *et al*., 2016). For proper metabolic functioning and improve pig's health, zinc is required (Prasad, 1991). Nano zinc oxide is required in contrast with traditional Zn sources, the beneficial findings of Nano zinc oxide are higher than the traditional zinc (Swain *et al*., 2016). As opined by Abdelnour, *et al.* (2021) and Mittal, *et al*. (2020), the several positive attributes of Nano zinc in animal production, immunity and relieve in reproductive disruption will aid in food security with the use of better, conventional farming methods that have less if not any negative environmental effects. Despite the reservation in adoption of Nano zinc oxide owing to its toxicity (Sharma, *et al*., 2012, Wang, *et al*., 2006), it was confirmed that Nano zinc oxide still have a lot of potential in agriculture, such as feed additives (Sabir, *et al*., 2014). Adequate levels of Nano zinc in the diet would go a long way to minimize piglet mortality, maintain better animal performance, and have high antibacterial efficacy with low toxicity in eukaryotic environments (Berube, 2008). There are no or limited

studies and articles showing that the effect of different inclusion levels of Nano zinc oxide on Large White weaner piglets. The option of using Nano technology has not been widely exploited although there is no ban in some parts of Africa especially South Africa, hence this study aims to fill this gap

1.3 Research aims

The aim of the study was to investigate the effects of dietary inclusion levels of Nano zinc oxide on growth performance, blood biochemical parameters, and carcass and meat quality in weaner piglets, as alternatives to Tylosin 10%.

1.4 Specific research objectives

- To evaluate the effect of dietary inclusion of inclusion levels of Nano zinc oxide on the weekly weight gain, overall weight gain and total body weight of weaner piglets
- To determine the concentration of blood parameters (albumin, creatinine, total protein, aspartate aminotransferase, alanine transaminase, phosphorus ions, calcium, iron, alpha and beta globulin) of pigs fed diets with graded levels of Nano zinc oxide,
- To evaluate the carcass characteristics (back fat thickness, hot and cold dressed mass) and meat quality parameters (meat colour, water holding capacity, drip loss, cooking loss) of pigs fed diets with graded levels of Nano zinc oxide
- To evaluate the GIT morphology of pigs fed diets with graded level of Nano zinc oxide.

1.5 Hypothesis

$H_{o:}$ There were no significant differences in weekly weight gain, overall weight gain and total body weight of weaner piglets fed diets with graded levels of Nano zinc oxide.

$H_{o:}$ There were no significant differences in blood parameters (albumin, creatinine, total protein, aspartate aminotransferase, alanine transaminase, phosphorus ions, calcium, iron, alpha and beta globulin) of weaner piglets fed diets with graded levels of Nano zinc oxide

$H_{o:}$ There were no significant differences in the carcass characteristics (back fat thickness, hot and cold dressed mass) and meat quality parameters (meat colour, water holding capacity, drip loss, cooking loss) of piglets fed diets with graded levels of Nano zinc oxide.

H_{o}: There were no significant differences in the GIT morphology of pigs fed diets with graded level of Nano zinc oxide

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Chapter 2

LITERATURE REVIEW

2.0 Introduction

Pork accounts for approximately 40% of the world's meat consumption (McMichael, *et al*., 2007). The pig sector will always play an imperative role in meeting the global demand for meat, owing to the rapidly increasing need for meat production and the projected need for a 20% increase in global feed production (McMichael, *et al*., 2007). Raising pigs in the Republic of South Africa (RSA) plays an important role in farming systems as a protein source which also serves as a means of revenue for the family. Pigs can be raised in a free-range system, semi-intensive system, or intensive farming system (Compassion in World Farming [CIWF], 2013).

Farmers use corn or soybean meal as the main ingredient in pig diet (Hoffman & Baker, 2011). Tusnio, *et al*. (2017) highlighted that at the moment in Europe, soybean meal which is considerably optimised in farm animal diets is a sufficient source for protein requirements. Furthermore, it was opined that other protein sources have to be researched as a result of the global surge in prices (Tusnio, *et al*., 2017). The zinc in the animal feed industry is commonly sourced from zinc oxide and zinc sulphate (Wedekind & Baker, 1990). As an in-feed source, the Nanozinc oxide offers the advantages of a high bio-availability, a small, stable additive dose, and a strong response in comparison to other elements, etc. Nano zinc oxide as a feed additive can effectively supplement zinc animal demand, encourage animals to grow well, and improve feeding efficiency, mitigate diarrhoea effectively, and prevent causing antibiotic drug residue on the by-product of livestock (Swain, *et al*., 2016). Due to the antimicrobial growth promoters

being banned in a lot of countries, high dietary doses have received great attention from both farm workers and researchers (Long, *et al*., 2017). At doses between 2000 and 4000 mg/kg feed which are known to be of concern for pollutant potential animal waste, Zinc oxide is currently successful in improving the efficiency and intestinal health of weaned piglets (Bonetti, *et al.*, 2021).

The early-life microbial colonization with potentially beneficial and diverse gut microbes can influence the maintenance of intestinal homeostasis and prevent gut symbiosis (Pluske, *et al*., 2018; Nowland, *et al.*, 2019). Jha, *et al*. (2019) stated that dietary fibres can modulate the gut microbiome and influences the gut health nutritional changes. According to Sugiharto, *et al*. (2014) and Campbell, *et al.* (2013), social changes and environmental changes expose piglets to different pathogens in their feed and the environment just as the passive immunity conferred by the antibodies in the sow's milk has reduced. Changes in the microbial community in the gut may translate to an imbalance in the gut microbial ecosystem, leading to intestinal disorders (Cheng, *et al*., 2011). Besides weaning related stress, another factor responsible for this imbalanced microbiota is the presence of enterotoxigenic *Escherichia coli (ETEC)* in the gut of piglet (Hinton, *et al*., 1985).

Feeding piglets with increased doses of zinc oxide leads to excess zinc that cannot be absorbed being leached to the environment and consequently, increasing the risks of multiple drug resistance and heavy metal contamination (Long, *et al*., 2017). Researchers have tried to decrease the usage of conventional zinc oxide in pig feed but improve its biological effects especially in diarrhoea (Long, *et al*., 2017). Currently, changing the conventional zinc oxide powder into porous particles or Nanoparticles is a common practice (Long, *et al*., 2017). Water bodies can also be affected by organic effluents and pathogens present in manure.

Environmental stresses related to pig processing are being eased with technical advancement (e.g. in the field of housing facilities, compost, storage, and options for energy generation units), and management activities (e.g. modifying feed quality and compost spreading methods). Such improvements as in-feed compositions will give all farmers a win-win situation where both production costs and environmental risks are minimized (Parris, 2011). Nanotechnology, modern matrices such as biopolymers, and encapsulation deliver future technologies that will mitigate the complexities of piglet weaning (Barreto & Conte- Junior, 2018).

2.1 Nanotechnology

According to Swain (2015), Nanotechnology is used in the production of new or substantially enhanced properties owing to its nano-scale products with a minimum one scale for production of material, equipment, or systems. In easy terms, it refers to the particles with size ranging from 1-100nm. According to Sarathi & Thilagavathi, (2009), Nanotechnology "is the art and science of manipulating matter at the Nano scale to create new and unique materials and products. Pokropivny, *et al*., (2007) indicated that even though nanotechnology is a recent development, it can be said that it has existed for centuries. The Chinese used gold Nanoparticles as an inorganic dye for the making of their ceramics and artefacts more than a thousand years/ ago. They further identified that Nano science is the combination of our ability to see and manipulate matter on the Nano scale with the understanding of atomic-scale interactions. Bhushan (2017) also defined Nanotechnology as any technology on a Nano scale that has applications in the real world. Also, Nanotechnology encapsulates the production and application of physical and biological systems at scales ranging from individual molecules. Summarily, Nanotechnology can be defined as the

science of studying small things with unique properties because of the small size (Bayda, *et al*., 2020).

2.2 Properties of Nanominerals

Hochella, *et al*. (2008) reported that the Nano minerals in most living organisms are natural and abundant throughout the atmosphere, seas, groundwater, and surface water. Moreover, in crustal or mantle rocks, Nano minerals are more constrained and can impact deeper earth processes. "The intrinsic properties of Nano metals are defined primarily by their size, shape, composition, crystalline structure, and morphology, Dickson and Lyon (2000) said." it has been also noted that Nano minerals are strongly affected by their particle size in the activity of functional behaviour including chemical, catalytic or biological effects (Lewis & Klibanov, 2005; Rosi & Mirkin, 2005; Swain, *et al*., 2015).

Under high temperatures and strain, Nanoparticles are stable. Owing to their small scale, they will quickly be ingested through the gastrointestinal tract and becoming more productive at smaller levels than larger amounts of other components. In the animal body, because of their broader surface range, Nano minerals interact with organic and inorganic materials more efficiently. The impact of Nano zinc particles on human cells has also been smaller. Small intestines are circumvented and spread widely in blood, brain, lung, heart, kidney, spleen, liver, intestines, and the stomach (Swain, *et al*., 2016).

2.3 Nano mineral feeding in livestock

The application of Nanotechnology in the production of Nanoparticles which can be used in improving digestion and absorption in livestock cannot be overemphasized. The potential of

improving livestock production and animals, in general, can also be attributed to employing Nanotechnology. Nano minerals feeding in livestock have a significant growth-promoting and anti-bacterial effect. Nano minerals also enhance the reproduction and immunity of livestock (Swain, *et al*., 2015). Most abortion causes in animals can be examined with Nano sensors while Nano-antioxidants can prevent placenta and other reproductive complications following calving, thus improving problems related to infertility. Doses of range 2000 - 4000 mg Zn/kg of feed have shown signs in the improvement of performance and intestinal health in weaned piglets, though the doses are taken to be of concern as potential pollutants in animal wastes (Barreto & Conte-Junior, 2018).

Anti-bacterial agents are paramount in destroying infectious diseases (Bhagyaraj & Oluwafemi, 2018), however, with their wide use and abuse; the growth of bacterial resistance to anti-bacterial drugs has become a concern. Engin and Engin (2019) stated that a high dose of administration of antibiotics executes resistance and leads to unendurable toxicity. This has brought an urge to develop alternative strategies to treat bacterial diseases. Anti-microbial Nanoparticles are among those that have shown significance in treating infectious diseases, including those that are resistant to antibiotics, *in vitro* as well as animal models (Mohammed, *et al*., 2012).

The emergence of antibiotic-resistant bacteria is recognized as a crucial problem for public health (Serwecińska, 2020). Destruction of antibiotic-resistant bacteria requires multiple expensive drugs that may have side effects. Consequently, treatments are costly and require more time. Nanoparticles offer a new way to combat multidrug-resistant bacteria (Mohammed, *et al*., 2012).

Nanoparticles such as copper oxide, nickel oxide, zinc oxide, and antimony (iii) oxide are used against *E-Coli, B.Subtilis,* and *S.aureas.* CuO Nanoparticles have the highest toxicity followed by zinc oxide (except for *S.aureas*), NiO, and Sb_2O_3 Nanoparticles. The toxicity of ions, which come as a result of Nanoparticles depends on the natural toxic properties of heavy metals (Mohammed, *et al*., 2012). The antibacterial activities of Nanoparticles depend on:

- Physiochemical properties of the Nanoparticle.
- Type of bacteria

2.3.1 Effect of the zinc oxide Nanoparticles Supplementation on Biological Systems

2.3.1.1 Growth

Zinc particles have been reported to enhance growth performance and improve feed utility and provide an economic benefit in weaning piglets and poultry (Swain, *et al*., 2016). Lina, *et al*. (2009) used 40ppm of Zinc as Nano zinc in broiler diets and there was a significant increase in growth performance of poultry. Gark, *et al*. (2008) also observed a notable improvement in the average daily gain and feed efficiency in lambs given zinc methionine as compared to a group that was supplemented with zinc sulphate ($ZnSO_4$). Contrary to other findings, Nunnery, *et al*. (1996) highlighted that the average daily gain on steers supplemented with zinc sulphate was 7.4% greater than those supplemented with zinc methionine but consecutively, feed intake and feed efficiency were uniform. The retardation of bone growth due to deficiency of Zn also proves its importance in the growth and mineralization of bone tissues (Liang, *et al*., 2012).

2.3.1.2 Milk production

In cows with subclinical mastitis, Nano zinc oxide has been reported for decreasing the somatic cell counts and increasing the quality of milk in contrast to traditional sources of zinc-oxide, which means Nano zinc oxide, can be used to avoid and treat subclinical mastitis in cows (Rajendran, *et al*., 2013; Swain, *et al*., 2016).

2.3.1.3 Rumen fermentation

Nano zinc oxide supplementation was commended to promote the development of the ruminal micro-organism; to improve the ruminal microbial protein synthesis and improve energy production in early incubation phases (Cheng, *et al*., 2011). The volatile concentration of fatty acids was also greatly increased also the production of microbial raw protein and the fermenting of organic materials at 6 and 12 hours of incubation time. Nano particles have a detrimental effect in the concentration of ammonia nitrogen and the ratio of acetate to propionate (Swain, *et al*., 2016).

2.3.1.4 Immunity

Zinc deficiency decreases immune responses and susceptibility to infections both in humans and in animals (Ao, *et al*., 2011). Zinc is essential for the structure and operation of various enzymatic, cellular, and regulatory proteins (Frederickson, *et al*., 2000; Swain, *et al*., 2016). In 400mg/kg zinc oxide Nanoparticles, diarrhoea was decreased by 49.1% compared to 21.6% in comparison to 3000mg/kg of zinc oxide (Swain, *et al*., 2016).

2.3.1.5 Reproduction

Nano-sensors will research abortion causes (Swain, *et al*., 2016). One field which needs to be explored to prevent the occurrence of placental retention and other reproductive problems following calving and the improvement of infertility problems is the Nano antioxidant. In scavenging species of reactive oxygen, zinc has anti-oxidant properties and is essential (Swain, *et al*., 2016). The lack of zinc may lead to increased oxidative damage, which can cause low quality sperm (Colagar, *et al*., 2009).

Zinc controls energy intake by Adenosine Triphosphate mechanism that contracts and regulates the reserves of energy for the phospholipids and thus affects sperm motility. Zinc also regulates goat sperm motility by affecting sperm flagella production. Ahmed, *et al*. (1997) stated that in buffalos, high zinc levels in feed is important for sperm and iodine viability and male infertility, and possibly as a result of poor zinc feeding, low sperm content. It was also highlighted by Campbell and Mills (1979), Najafzadeh, *et al*. (2013) that high incidents of abortions and still-births are a result of diets that have an insufficient amount of zinc.

2.3.1.6 Anti-bacterial activity of Nano zinc Oxide

Anti-bacterial agent refers to the reagent that destroys or inhibits the growth of bacteria without being harmful to the surrounding tissue. According to Arabi, *et al*. (2012), zinc oxide Nanoparticles are effective against spores which makes it resistant to high-temperature, high-pressure infection, gram-positive bacteria, and gram-negative spores. As bacteria are treated with Nanoparticles of zinc oxide, their permeability greatly increases, which prevents proper transport through the plasma membrane leading to cell death (Auffan, *et al*., 2009).

Zinc oxide Nanoparticles' anti-bacterial behaviour depends on surface area and concentration; although they have no effect on the crystalline form and particle form (Swain, *et al*., 2016). Zinc oxide Nanoparticles have excellent anti-microbial properties, and they are suitable for mixing with feed as a growth-promoting agent or for stopping diseases from occurring (Swain, *et al*., 2016).

2.3.1.7 Toxicity of Nano zinc

Zinc toxicity in liver, spleen, heart, and pancreases can be quarry on oral exposure (Swain, 2016). The toxicity of Nanoparticles in cells is caused, resulting in the formation of free radicals that are responsible for oxidation, inflammation, and cell death (Xia, *et al.*, 2008). Zinc oxide particles appear to aggregate in the liver tissue and cause significant renal losses (multifocal intestinal nephritis in 75% of animals) as well as toxicity (oedema and hepatocytes degeneration) (Swain, *et al*., 2016).

2.4 Preparation of Nanoparticles

The preparation of Nanoparticles aims at having a pre-eminence of better control over the size, morphology, purity, quality, and quantity of the product. There are several methods that can be used to prepare Nano minerals which are:

2.4.1 Physical methods

Evaporation, condensation, utilizing an atmospheric pressure tube furnace and laser ablation combined, electric arch discharge, chemical deposition of vapour, gaseous synthesis, and ball-friction-removal process, physical vapour deposition, can be used for the preparation of nominal materials. Ball moulding is used to mould material into very fine Nano size powder to be used in

animal feeding. The lack of solvent degradation and the complete Nano mineral regeneration are the benefits of synthesizing Nano materials using a physical approach (Swain, *et al*., 2015).

2.4.2 Chemical methods

The Nanoparticles are mechanically generated with more particle sizes, but the chemical method enables the development of a uniform particle size such that effective, regulated bulk can then be created by the chemical method (Swain, *et al*., 2015).

There's still a risk of toxicity when chemical syntheses use dangerous compounds. In the synthesis of Nano substances are organic, non-toxic compounds such as glucose, sugar, amino acids, and herbal extracts (Swain, *et al*., 2015). Sonochemical and microwave synthesis is developed to a large degree and often cheaper are other methods other than toxic chemical synthesis (Swain, *et al*., 2015).

2.4.3 Biological methods

Attention is based on an earth-friendly synthesis of Nano materials that seem simple, less toxic, and effective than traditional methods that require more resources and are dangerous. Biosynthesis techniques used by plant extracts and biological microorganisms have been used as an alternative to physical and chemical approaches for the development of Nano minerals. The processing of Nano mineral particles, through the use of viruses, plants, algae, fungi, and bacteria, has been cost-effective and nontoxic. By adding many plant materials, by bio-synthesizes of Nanoparticles from zinc Oxide in the early years, the biological methods have compiled numerous metal Nanoparticles such as silvers, gold, palladium, cadmium, selenium, titanium, and titanium. The use of plant materials for the compilation of Nano minerals is easier and more beneficial, as their synthesis has only one step, fast reclamation from final solutions,

environmentally sustainable and cost-effective processing, less time, economical sustainability, and no need for preservation of culture (Swain, *et al*., 2015).

Biosynthesis of the Nano-based structure is particularly very handy for medical applications as they provide cost-effective, non-toxic, and environmentally friendly materials. Organic sources are employed for the making of Nano materials which thwarts the necessity for using chemicals that are toxic as reducing agents. Plants are considered among the most resourceful sources for the synthesis of the Nanoparticles as they host a variety of metabolites which act as both reducing and stabilizing agents (Agarwal, *et al*., 2018).

Zinc oxide particles have been made from different plant materials like *Azadirachra indica, Gamella sinensis, Rosa canina, Moringa oleifoera, Vitex negundo, Ocimum basilicum, Solanum, Pongamia pinnata, Phyllanthus niruri, Trifolium pretense and Hibiscus subdariffa*. Biosynthesized zinc oxide Nanoparticles possess more antifungal activity than commercial drugs (Vijayakumar, *et al.* 2020).

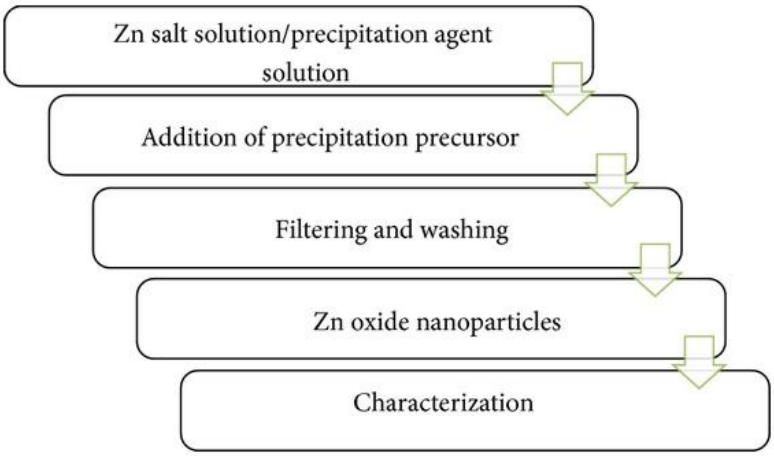

Figure 1: The characterization of Nano-Zinc oxide synthesis

Source: Sabir, *et al*. (2014)

2.5 Application of Nanotechnology and Nanoparticles

In South Africa, potential applications of Nanotechnology are still in the pipeline. Evidently, the results are not far from materialization with potential benefits not only for the economy of South Africa but also to improve the lives and livelihood of the ordinary people which are still not assessing the basic amenities such as clean water, electricity and proper health care. Many industries in South Africa are working with the government and the academia to open doors of potentials and opportunities for Nanotechnology development and application. Npep, (2017) highlighted that the Science and Technology Department of South Africa initiated the National Nanotechnology Policy in 2005 intending to organize national Nano research and development. This focuses and extends on six areas: water, electricity, sanitation, agriculture, chemical and bioprocessing, mining and minerals, and advanced materials and development.

2.5.4 Agriculture

It is safe to say the introduction of Nanotechnology into agriculture can change the whole industry from generation to preservation, handling, and transportation of products even the wastes generated from farms. Rashidi and Khosravi-Darani, (2011) stated that the challenges of low profit in cultivable regions, large uncultivable ranges, shrinkage of cultivable terrains, and wastage of inputs like water and food security for developing numbers can be tended through different utilizations of Nanotechnology. Table 1 shows the various applications of Nano zinc oxide and their effects on different systems of investigation.

Table 1: Various applications of Nano zinc oxide and their effects on different systems of investigation

Species	**Systems under investigation**	**Effect and conclusions**	**References**
Rabbit	Reproduction	Increased semen content, the overall concentration of live sperm, percent of sperm motility, heat-stressed rabbit's conception rate.	El-Masry, *et al*. (1994)
Human, lab animals	Immunity	Reduced Zn-deficient immune systems and infection tolerance.	O'Dell and Sunde, (1997)
Goats	Immunity	Enhanced udder resistance to Zn supplements for dairy goats.	Salama, *et al*. (2003)
In Vitro and *in vivo*	Antioxidant	It Plays an antioxidant-like in vitro impact. At in vivo pharmacological doses, Zn is safe against pro-oxidizing agents, and a dietary Zn deficit is vulnerable to oxidative damage in cells by shielding sulphydryl	Bray and Bettger, (1990)

		classes.	
Ruminants	Reproduction	Increased frequency of Zn deficient ewes abortions and mortality	Campbell and Mills, (1979), Najafzadeh, *et al*. (2013)
Poultry (Broilers)	Growth, carcass traits, and meat quality	Increased ADFI, ADG, DM and intramuscular muscular fat ratios, percentage of eviscerated muscle production, breast muscle redness and thigh pH values, reduced thigh muscle shear strength, breast, and thigh muscle dropouts.	Liu, *et al*. (2011)

Source: Swain, *et al*. (2016)

2.6 Essential nutrients for pigs

The proficiency and productivity of a piggery are very sensitive to the feed used in production. According to the Department of Agriculture and Fisheries, (2010), to achieve profitable result, pigs need to be fed diets that satisfy their needs to reach their potential for lean meat growth. To optimize production in pigs, nutrients need to be provided in balanced amounts and at levels that placate the pig's requirements for maintenance, growth, and reproduction.

Pigs require a number of essential nutrients to meet their needs for maintenance, growth, reproduction, lactation, and other functions. According to Cromwell, (2018), pigs require 6 general classes of nutrients: water, carbohydrates, fat, protein (amino acids), minerals,

and vitamins. The Department of Agriculture and Fisheries highlighted that the nutrient requirements for various bodyweight groups of pigs from 5 – 135 kg body weight

2.6.1 Water

Water is the most important ingredient of all. Water is required to maintain body temperature, transport digested food throughout the body, and also help remove the wastes from the body of the pig. From the weaning stage, pigs should have free access to water. Cromwell, (2018) discussed that the amount of water required by pigs varies with age, type of feed, environmental temperature, the status of lactation, and many more factors. Furthermore, it is highlighted that a growing pig will require about 2 – 3 kg of water for every kg of dry feed consumed. While in the case of a lactating sow, a larger quantity of water is required because of the high-water content of the milk they produce.

2.6.2 Carbohydrates

Carbohydrates are essential in an animal's body as they are the primary source of energy that provides a minimum of 50% of the total cost of pig production. They affect digestion and the other functions of the gastrointestinal tract. Most of the energy requirement for pigs is met by fats and carbohydrates (Pettigrew, *et al*., 1991).

2.6.3 Fat and oil

Fats and oils are sources of energy (Pettigrew, *et al*., 1991). Fats and oils provide energy approximately 2.25 times higher than carbohydrates (Lauridsen, *et al*., 2007). The use of fats and oils in pig diets is of great importance because they affect animal performance (Lauridsen, *et al*., 2007) and also have the capability to reduce the dietary heat augmentation in growing and finishing pigs during times of environmental heat stress (Park, *et al*., 2012)

2.6.4 Protein (Amino Acids)

DAF, (2010) discussed that out of the 20 different types that occur in chemical blocks of protein, a pig needs only 8 or 9 of these in its diet which are the "essential" amino acids. All amino acids must be present in their correct balance for protein synthesis to occur in the pig, which is presented in table 2.

Table 2 shows the ideal amino acid patterns for growers and breeders.

Amino acid	**Weaners 5 – 20kg**	**Growers 20 – 50kg**	**Finisher 50 – 100kg**	**Lactating pigs**	**Pregnant pigs**
Lysine	100	100	100	100	100
Isoleucine	57	57	57	57	57
Methionine	30	30	30	30	30
Methionine + cysteine	55	60	65	55	55
Threonine	63	67	70	60	60
Tryptophan	18	18	18	18	18

Source: DAF (2010)

2.6.5 Minerals

This is essential to maintain the animal's bone structure and regulate many biochemical processes. This can be gotten from grains and protein sources and primarily from mineral supplements. Lammers, *et al*. (2007) highlighted that pig require calcium, phosphorus, and sodium as macro-minerals. Further minerals include Iron, Zinc, Copper, and Manganese in trace amounts.

2.6.6 Vitamins

Vitamins in the animal body are required in regulating many biochemical processes. Most vitamins can be found in the feedstuff for the pigs. Cromwell, (2018) stated that Vitamin A, Vitamin D, Vitamin E, Vitamin K, Riboflavin, Niacin (Nicotinic acid), Pantothenic acid, Vitamin B_{12}, Thiamine, Vitamin B_6, Choline, Biotin, Folacin and Ascorbic acid (Vitamin C) are all the required Vitamins for a pig.

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CHAPTER 3

EFFECT OF NANO ZINC OXIDE SUPPLEMENTATION ON THE GROWTH PERFORMANCE OF WEANER LARGE WHITE PIGLETS

3.1 Abstract

This study evaluated the effect of dietary Nano zinc oxide on the growth performance of weaner Large White piglets. A total of thirty (30) piglets with an average body weight of 7.6±0.54 kg were randomly assigned to 5 different treatments: 600, 400, 200 mg Nano zinc oxide/kg DM, positive control (100 mg Tylosin 10%), and the negative control (no additive) supplemented in a completely randomized design. Data were collected weekly and analysed in a completely randomized design. The results showed that supplemented levels on Nano zinc oxide statistically ($P<0.05$) influenced the total weight gain and feed conversion ratio of the pigs across the treatments. Piglets supplemented with 200mg/kg had the highest mean values for weight gain and daily weight gain and those fed 600 and 400mg/kg had similar values while the control group had the least comparable weights. Also, the 200mg and 600mg/kg gave the best comparable feed conversion ratio compared to others. However the negative control and the positive control resulted in poor FCR with no influence on the intake across the treatment groups. Summarily, the supplementation of Nano zinc oxide improved the growth performance of weaner pigs with better average daily gain.

3.2 Introduction

Zinc is an important mineral with many functions in livestock animals, it aids nutrient

metabolism, as a component of numerous metalloenzymes; appetite control, regulation of the immune system, oxygen-free radical scavenging, and transcription factors (Fathi, *et al*., 2016). It is further indicated by Liu, *et al*. (2011) that zinc enzymes are involved in the breaking down of carbohydrates, lipids, proteins, nucleic acids and encapsulates all known classes of enzymes.

Zinc's impact on product quality has been documented to differ from different sources, organic or inorganic (Zhao, *et al*., 2014). The bioavailability of organic Zn is higher than that of inorganic Zn, but its higher cost limits the use of organic Zn in animal diets (Zhao, *et al*., 2014). In animal production, due to the low utilization rate of inorganic Zn, the additional concentration of inorganic Zn is 20-to 30-fold higher than the normal requirement to meet the animal needs (Bratz, *et al*., 2013). High dietary Zn, however, can lead to excess in the stool, causing contamination of the environment. In addition, high Zn supplementation may affect the body's balance of other trace elements and decrease the stability of vitamins and other nutrients, and long-term applications may cause zinc residue in the animal body (Zhao, *et al*., 2014).

Recently, the development of Nanotechnology and its related products has progressed rapidly in various scientific fields; undeniably, this branch of science has affected human, animal, environmental, and industrial lives. In this regard, Nano-particulate zinc oxide (zinc oxide) has attracted a great deal of attention as Nano-particulate formulations exhibit novel qualities such as volume, shape, large surface area, high surface activity, high catalytic efficiency, and good absorbing ability (Wijnhoven, *et al*., 2009). Compared to zinc oxide, Nano zinc oxide has a stronger chemical activity and is associated with numeral organic compounds in oxidation reactions (Zhao, *et al*., 2014).

Nano zinc oxide can act as an antibacterial agent and enhance growth. Several studies have proven that the enhancement of growth performance in poultry and livestock depends on the

amount of dosage of Nano zinc oxide (Yusof, *et al*., 2019; Swain, *et al.*, 2016; Fathi, *et al*., 2016; Huang, *et al*., 2015). Swain, *et al*. (2016) highlighted that zinc oxide promotes growth performance; improves feed utility and provides economic benefits in weaning piglets and poultry.

Nano materials are particles below 100 nm in size that are stable at room temperature and pressure. It is convenient for the gastrointestinal tract to consume them, considering the impact of their limited size; thus, they are more competent than larger zinc oxide sections at smaller doses (Pei, *et al*., 2019). Nano materials merge more productively with inorganic and organic substances in the body of an animal because of their large surface area. They possess the ability to bypass the small intestine and further distributed into the blood, brain, heart, kidney, spleen, liver, intestine, and stomach. The particle size greatly influences the functional activities such as the chemical, catalytic or biological effects of Nanoparticles (Pei, *et al.*, 2019). Therefore, this study emphasizes that the supplementation of Nano zinc oxide will enhance growth performance of weaned pigs.

3.3 Materials and methods

3.3.1 Site description

The experiment was carried out at Velile pig broedery farms in North West Province. North West is a province with a climate that offers almost year-round sunshine, where warmer months are from August to March with average temperatures ranging from 22 to 34 ^{o}C and winter months are from May to July with temperatures ranging from 2 to 20 ^{o}C a single day. The province is located on longitude 25.64 and latitude -25.8 degrees east of the Greenwich, most of the land is flat and undulating with few high elevations. All parts of the province fall within the

summer rainfall area, with rainfall occurring between October and April. The protocol of this study was approved by the North West University Ethics committee with ethics number NWU-00735-18-A5 and was conducted in accordance with the ethics guidelines for the care and use of experimental animals.

3.3.2 Experimental arrangement and design

A total of 30 Large White weaner pigs aged between 21 - 23 days with an average weight of 7.6 kg ± 0.54 housed individually and were randomly allotted to 5 treatments, with each pen of size $3m^2$ x $6m^2$, each pen serving as replicate. Pigs were fed a basal diet with no additive (negative control), basal diet supplemented with powdered Nano zinc oxide (obtained from MK Impex Corp, Canada) at different levels of 200mg/kg, 400mg/kg, 600mg/kg and lastly 100mg/kg of feed additive that had Tylosin10% (Tylosin phosphate; positive control) procured at Virbac South Africa for 82 days. Pigs were offered a starter ration of 0.8kg per pig per day for 42 days and the remaining 40 days they were fed 1.6kg per pig per day (Palmer, 2019) until slaughter and had *ad libitum* access to water provided with nipple drinkers. The pigs were fed once daily between 10am and 11am. A footbath was provided at the entry point for biosecurity purposes and pens were thoroughly cleaned and disinfected on a daily basis. Animals were ear notched at the beginning of the trial for identification and tail docked for health and hygiene purposes.

3.3.3 Data collection

The daily feed intake was recorded and the weekly weights were recorded with a digital scale using formulae:

$$Average\ daily\ gain\ (Kg) = Initial\ weight\ (Kg) - Final\ weight\ (Kg)$$

$$Feed\ intake\ (Kg) = Feed\ offered\ (Kg) - Feed\ refusal\ (Kg)$$

The distribution of the treatments amongst the pigs is shown in Table 3, with the nutrient composition of the basal diet shown in Table 4.

Table 3: Treatment distribution

Pen number	Treatment
1	Basal diet + 600mg Nano zinc oxide
2	Basal diet + 400mg Nano zinc oxide
3	Basal diet + 200mg Nano zinc oxide
4	Basal diet + conventional premix Tylosin 100mg/kg (positive control)
5	Basal diet and no additive (negative control)

Table 4: Nutrient Composition of experimental Basal diets

Parameter	Weaner diet	Starter diet	Grower diet
Protein	190	180	150
Total Lysine	12	11	8.5,
Moisture	120	120	120
Fat	25	25	25
Fibre	40	50	80
Calcium	8-10	7.5-10	6-10
Phosphate	6.5	6.0	5
Limestone	1.30	1.30	1.30
Vit-min premix	0.04	0.04	0.04
L.threonine	0.04	0.04	0.04
DL.methionine	0.09	0.09	0.09
ME, MCal/KG	3.48	3.48	3.48

Crude Protein %	24.17	24.17	24.17
Calcium %	0.84	0.84	0.84
Phosphorus %	0.66	0.66	0.66
Lysine %	1.54	1.54	1.54
Methionine %	0.45	0.45	0.45
Cysteine %	0.39	0.39	0.39
Threonine %	0.96	0.96	0.96
Tryptophan %	0.28	0.28	0.28
Arginine %	1.60	1.60	1.60
Histidine %	0.67	0.67	0.67
Isoleucine%	1.03	1.03	1.03j
Leucine %	2.05	2.05	2.05
Phenylalanine %	1.21	1.21	1.21
Valine %	1.09	1.09	1.09

3.4 Statistical analysis

The weekly body weights were analysed using the repeated measures ANOVA PROC GLM, while the overall performance was done using general linear model procedure PROC GLM as contained in SAS software. Significant mean among the treatment groups were separated using the Duncan multiple range test as contained in the SAS package.

$$Y_{ij} = \mu + DA_i + E_{ij}$$

$Y_{ij} = \mu + DA_i + E_{ijk}$
Y_{ij} = dependant variable
U= overall mean
DA_i = effect of dietary additive
E_{ij} = random error associated with observation ij, assumed to be normally and independently distributed

3.5 Results

	treatments	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10	week 11	week 12	week 13
600mg nano zinc oxide	1	9,84	13,44	17,21	23,6	28,42	31,7	37,18	43,8	46,2	51,4	56	60	64
400mg nano zinc oxide	2	9,49	12,4	16,4	23,2	25,5	29	35	40	43	47	51	55	60
200mg nano zinc oxide	4	10,4	13,6	17,4	25,8	28,8	31	40	43	46	48	55	64	68
Tylosin 10%	5	10,6	13	17	25	27	37	43	47	51	54	56	59	62
Negtaive control (no additive)	6	9,3	12,9	15	22	24	29	34	40	43	46	50	54	56

Figure 3: Weight distribution of Pigs

Table 5: Growth performance characteristics of weaner pigs fed supplemental levels of Nano zinc oxide

[1]Parameters	-ve control NO ADDITIVE	+ve Control TYLOSIN 10%/kg	zinc oxideNano zinc oxide level 200mg/kg	400mg/kg	600mg/kg	SEM
FW (kg)	56.67^{c}	58.50^{c}	68.60^{a}	59.17^{bc}	64.4^{ab}	1.11
BWG (kg)	47.34^{c}	47.88^{c}	58.56^{a}	49.6^{bc}	54.56^{ab}	1.12
FCR	2.5^{a}	2.5^{a}	2.01^{c}	2.14^{ab}	2.2^{bc}	2.2
DG (kg*)	0.52^{c}	0.53^{c}	0.64^{a}	0.55^{bc}	0.6^{ab}	0.54

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

[1] FW = Final weight, BWG = body weight gain, ADFI = average daily feed intake, FCR =Feed conversion ratio, DG = Daily gain

The results in Table 5 shows that the supplemental levels of Nano zinc oxide significantly (P<0.005) increased the overall weight gains, weekly gains, daily gains and feed conversion ratios, the null hypothesis is rejected. The highest final weight gain was obtained in pigs fed 200mg/kg of feed Nano zinc oxide, which is comparable to those that were fed 600mg/kg Nano

zinc oxide while those fed the negative control and the positive control had the least values. The lowest daily weight gains were recorded from animals on both negative and the positive controls while those 200mg and 600mg/kg Nano zinc oxide supplementation had the best comparable daily gains. In addition, the 200mg and 600mg/kg gave the best comparable feed conversion ratio compared to others. However, supplementation of negative control and the positive control resulted in poor FCR. On the other hand, the supplementation of Nano zinc oxide had no significant influence ($P>0.05$) on the average feed intake.

3.6 Discussion

Studies indicated that Nano zinc oxide has a dose-dependent effect in animals and its supplementation can aid animal health development and growth. (Hongfu, 2008; Yang & Sun, 2006; Lien, *et al*., 2009; Mishra, *et al*., 2017; Sahoo, *et al*., 2014). Particles of Nano zinc oxide have been reported for increased productivity, improved feeding capabilities, and economic benefits in weaning of poultry and piglets (Yang & Sun, 2006; Mishra, *et al*., 2014).

Consistent with the results of this experiment are the results from an experiment conducted by Pei, *et al*. (2019), where incremental levels of Nano zinc oxide were supplemented in pig diets and improved growth performance was observed. Similarly, encouraging results in average daily gain was obtained by feeding basal diets supplemented with 200, 400, and 600mg/kg of Nano zinc oxide or 3000mg/kg conventional zinc oxide (Hongfu, 2008).

In consonance with the results of this study, Wang, *et al*. (2017) also reported that dietary supplements on Nano zinc oxide significantly increased the body weight gain and the average daily gain on weaned piglets but did not affect the average daily feed intake.

In another experiment by Pei, *et al*. (2019), supplementation of 1200mg/kg and 3000mg/kg of Nano zinc oxide was implemented in piglets, which also increased the weight gain and average daily gain. Long, *et al*. (2017) also compared the effects of the porous and Nanoparticles, he concluded that both Nanoparticles and porous particles of Nano zinc oxide improved growth performance. Wang, *et al*. (2017) further highlighted that 800mg/kg of Nano zinc oxide increased average daily gain but did not affect average feed intake and feed intake, this indicated that higher levels of Nano zinc could improve the growth performance characteristics of pigs. In a study by Cho, *et al*. (2015), they established that high doses at 2000-4000mg/kg of zinc oxide are added to nursery diets as a growth promoter, and this further buttress Nanozinc as a growth promoter as indicated from the result of this study. This result of the study is supported by the findings of Long, *et al*. (2017; Wang, *et al*. 2017 & Baretto and Conte-Junior 2018) that all found Nanozinc as growth enhancer in their studies.

The supplementation of Nano zinc oxide had a positive effect on the feed conversion ratio of pigs; however the negative and positive controls had a higher feed conversion ratio than the Nano zinc oxide supplementation. In a study by Van Lunen, (2003), it was concluded that the supplementation of Tylosin phosphate did not have any effect on the feed conversion ratio of pigs but rather gave the same results as the ones that were not supplemented with Tylosin which is in agreement with the results of this study. On another study by Bai, *et al.* (2019), it was suggested that the Nano zinc oxide materials improved the final body weight, average daily gain and feed conversion ratio which is in accordance with the results of this study.

3.7 Conclusion

It can be concluded that the use of Nano zinc oxide at doses of 200mg/kg and 600mg/kg can improve the daily and weekly weight gain with a better-feed conversion ratio.

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CHAPTER 4

DIETARY INCLUSION OF GRADED LEVELS OF NANO ZINC OXIDE ON THE CONCENTRATION OF BLOOD BIOCHEMICAL CONSTITUENTS OF LARGE WHITE PIGLETS

4.1 Abstract

This research evaluated the effect of Nano zinc oxide supplemented at different levels on the blood biochemical constituents of weaned pigs. Thirty (30) weaned pigs weighing an average of 7.6 kg ± 0.54 kg were used for the experiment and were offered supplemented levels of Nano zinc oxide at 200mg/kg, 400mg/kg, 600mg/kg, negative control (no additive), and 100mg Tylosin/kg feed as a positive control in an experiment that was arranged in a completely randomized design. Animals were bled and blood samples were collected at the end of the experiment and parameters such as albumin, total protein, phosphate ion, alpha and beta globulin, creatinine, calcium ions and iron ions were analysed from them. The blood levels of albumin, total protein, phosphate ions, alpha and beta globulin were significantly influenced ($P<0.05$) by the dietary inclusion levels of Nano zinc oxide. Blood creatinine, AST, ALT, calcium ions, and Fe were not influenced ($P>0.05$) by the dietary inclusion of Nano zinc oxide. Pigs fed 600mg/kg of Nano zinc oxide had the highest ($P<0.05$) albumin concentrations with the least observed in the groups fed 200 and 400mg/kg of Nano zinc oxide. Blood from pigs offered diets with Tylosin 10%, 200, 400 and 600mg/kg of Nano zinc oxide had significantly higher ($P<0.05$) concentration of phosphorus ions, alpha and beta globulins than pigs fed diets with no additives. It could be said that dietary supplementation of Nano zinc oxide at 600mg/kg increased some essential blood biochemical profile as well as protecting the immune system of the piglets.

4.2 Introduction

The welfare problems with the subsequent death of pig and piglets remain a significant issue in pig farming, however, today's pig industries are among the most mature and advanced sectors of modern agriculture that can aid in curbing the impending food security. The effectiveness of zinc in most studies has shown that Nano size medication and mineral absorption of total proteins have improved with Nano-sized medicines (Florence, *et al*., 1995; Desai & Banat, 1997; Davda & Labhasetwar, 2002; Win & Feng 2005). Lien, *et al*., (2009) documented that the bioavailable chromium and serum chromium levels of rats are greatly increased in chromium Nanoparticles. Tsai, *et al*., (2016) concluded that Nano-sized zinc oxide for dietary supplementation can increase zinc retention, carbonic anhydrase activity, growth performance, and serum zinc levels, and eggshell thickness in chickens.

Zinc is an essential nutrient that aids in the metabolism of lipids, carbohydrates, oxygen, and hormonal development and secretion (e.g. growth hormones, insulin, and sexual hormones). Zinc also influences the immune system's functioning, as zinc can influence thymus secretion of the thymulin, which activates the development of T-cells. A zinc deficiency can therefore lead to withering that causes problems with immune function (Brody, 1994; Wellinghausen, *et al*., 1997; Mocchegiani, *et al*., 1998a). Zinc also plays a role in comitogenic action by stimulating the immune response of piglets through incorporating zinc oxide phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) (Wellinghausen, *et al*., 1998a; Mocchegiani, *et al*., 1998b), as zinc may enhance the production of ghrelin by the stomach, which in turn will induce growth hormones and insulin secretion. This study therefore hypothesizes that supplementation of Nano zinc oxide will improve the blood biochemical constituents of growing pigs with subsequent effect on their overall health performance.

4.3 Methodology for blood biochemical parameters

Blood samples from 30 pigs (7ml each) were collected between 8am and 9am before feeding at the end of every week for biochemical studies according to Ekenyem and Madubuike (2007) and Okeudo, *et al*. (2003) with minor alterations. Blood was obtained by puncturing the jugular vein. Before the blood analysis was performed, blood smears were evaluated for possible clotting and coagulated samples were discarded. Approximately 7ml of blood from every pig was collected into a red top vacutainer tube without anticoagulant for blood biochemical analysis. After collection, the blood was left at ambient temperature for approximately 30 minutes to coagulate while being transported from the farm to the university research lab. The tubes were then centrifuged at room temperature for 10 minutes at 3000rpm to obtain serum. The serum was then frozen at -4^{o}C. The biochemistry parameters (Albumin, creatinine, total protein, aspartate aminotransferase, alanine transaminase, phosphorus ions, calcium, iron, alpha, and beta globulin) were analysed using the Idexx Catalyst One Chemistry Analyser.

4.3.1 Statistical Analysis

The data were analysed using general linear model PROC using SAS version 10. The significant mean among the treatment groups was separated using the Duncan multiple range test in the SAS package. The model is shown below.

Y ijk = μ + DA i+ E ijk

Y ijk = dependant variable

U= overall mean

DA i = effect of dietary additive

E ijk = residual error

4.4 Results

Table 6 presents the serum biochemical chemistry of weaned pigs fed incremental levels of Nano zinc oxide. Albumin, total protein, phosphate ions, alpha globulin, and beta globulin were significantly ($P<0.05$) influenced by the supplemented Nano zinc oxide, thus the null hypothesis is rejected. On the other hand, creatinine, AST, ALT, Calcium ion, and Fe were similar ($P>0.05$) across the treatment groups. Pigs fed 600mg/kg of Nano zinc oxide had the highest albumin concentrations with the least values observed in 200 and 400mg/kg groups. Pigs offered diets containing Tylosin and 600mg/kg of Nano zinc oxide had comparable higher total protein, while those fed the diets with no additive had the lowest total protein concentration. Higher similar Phosphorus ions were recorded in Tylosin and Nano zinc supplemented groups compared to the control group. This same trend was observed for alpha and beta globulins.

Table 6: Effect of diets containing levels of Nano zinc oxide on Blood biochemical parameters

	-ve control	+ve Control	Nano zinc oxide level			RI	SEM
Parameters	**No additive**	**Tylosin 10%**	**200mg**	**400mg**	**600mg**		
ALB (G/dL)	3.81^{cb}	4.14^{b}	3.51^{c}	3.40^{c}	4.72^{a}	**19-42** (G/dL)	0.93
CREA (FLMOL/L)	1.59	1.58	1.73	1.74	1.54	**77-165** (FLMol/L)	0.56
TP (G/DL)	6.88^{d}	8.13^{ab}	7.22^{cd}	7.72^{b}	8.66^{ab}	**52-83**(G/dL)	0.12
AST (IU/L)	100.38	102.56	91.69	98.53	98.56	*19.11-104.90*(IU/L)	3.69
ALT (IU/L)	10.32	10.34	10.18	10.28	10.54	**15.1-46**(mg/dL)	0.106
Pi (mg/dL)	9.24^{b}	11.74^{a}	11.31^{a}	12.14^{a}	12.62^{a}	*7-18*(mg/dL)	0.308
Ca^{++} (mg/L)	10.63	11.11	10.87	11.12	11.01	*2.20-3.80*(mg/L)	0.234
Iron (UMOL/L)	17.83	17.89	17.51	17.90	17.72	**9.9-29.8**(UMOL/L)	0.377
α-Globulin (g/L)	7.70^{b}	10.07^{a}	10.39^{a}	10.90^{a}	11.78^{a}	**7.7-13.7**(g/L)	0.312
β-Globulin (g/L)	8.85^{b}	11.58^{a}	11.94^{a}	12.55^{a}	13.55^{a}	**9.9-18.9**(g/L)	0.359

Means with different superscripts within row are significantly different (P<0.05), RI: reference intervals. AST- Aspartate aminotransferase, ALT- Alanine aminotransferase. *Liu & Liu, (2021)*. **Elbers *et al.*, (1994)**

4.5 Discussion

Serum albumin has been reported as the main protein of the blood plasma that is important in regulating blood volume through the regulation of the colloid osmotic pressure in the blood

compartment (Farrugia, 2010). The highest value of albumin observed for pigs fed 600mg/kg Nano zinc corroborate the results of Shinde, *et al.* (2006) who observed a higher albumin level in all zinc (irrespective of their sources) supplemented diets fed to guinea pigs compared to the control (with 0% Zn). In contrast to our observations in this study, Uniyal, *et al*. (2017) reported no differences in the albumin levels of pigs fed diets containing both organic form and Nano particle sized zinc. The increased albumin level observed in this study might be related to protein synthesis function of Zinc as influenced by the high reactivity of the supplemented Nano zinc. Zinc also functions in protein synthesis and can be related to albumin, which serves as the main protein of the blood plasma. However, more than half of all serum zinc is bound to albumin (Tsutsumi, *et al*., 2014). The bioavailability trait of Nano zinc availed more protein for the blood plasma, which sequentially produces a high level of albumin. This, in turn, gives more room for serum zinc bonding, blood volume regulation, and transport of molecules of low water solubility.

In relation to this study, Shinde, *et al.* (2006) reported higher total protein in all zinc-supplemented diets fed to guinea pigs compared to the un-supplemented group. On the other hand, Yalcinkaya, *et al.* (2012) reported a value of 3.96 g/dl of total protein of broilers fed a diet containing 80 ppm organic zinc source, which is lower compared to the values obtained in this study. The difference in total protein might be due to the reduced adsorption capacity of traditional (organic or inorganic) zinc oxide compared to the Nano zinc particle utilized in this study, or it might be species-specific, as seen by the lower total protein found in broilers. Uniyal, *et al*. (2017) also observed no significant difference in the total protein of pigs fed diets containing both organic form and Nano particle sized zinc which is in contrast to the result obtained in this study. The observed disparity can be because of the lower dosage (20mg) of Nano zinc employed in their study. High total protein observed in this study can be said to be

influenced by the supplemented Nano zinc. This further buttressed the high absorbing trait of Nano zinc which is made available to serve its function in protein synthesis and make protein more available in the blood.

Li, *et al*. (2016) observed higher globulin levels when Nano zinc is fed to pigs (though not differentiated into either alpha or beta globulin), this is however in line with the result of this study where higher levels of alpha and beta globulin. Conversely, Uniyal, *et al*. (2017) observed similar globulin levels in pigs fed diets containing both organic form and Nano zinc particle. The high level of globulin recorded in this study can be attributed to zinc supplementation, which has a stimulatory effect on the immune system in pigs (Rupic, *et al*., 1998). This indicated that supplementation of Nano zinc at the rate of 600mg/kg resulted in better immune system balancing and/or normative immune functions than other inclusion levels (200 and 400mg) and both controls as reflected in the globulin level in this study. It is sufficient to say, high serum levels of globulin following Nano zinc supplementation indicated its positive influence on immunity through stimulation for the production of the thymic hormone as well as maintenance of activities of other lymphoid organs (Borah, *et al*., 2014).

4.6 Conclusion

The inclusion of Nano zinc oxide did not affect the concentration of creatinine, AST, ALT, calcium and iron, therefore the null hypothesis is accepted. The null hypothesis is rejected in the case of serum albumin, total protein, phosphate irons, and alpha and beta globulins due to the significant differences in the means.

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CHAPTER 5

EFFECT OF DIFFERENT INCLUSION LEVELS OF NANO ZINC OXIDE ON THE CARCASS, MEAT QUALITY CHARACTERISTICS AND THE INTESTINAL MORPHOLOGY OF WEANED LARGE WHITE PIGLETS

5.1 Abstract

The objective of this study was to determine the effect of dietary Nano zinc oxide inclusions at different levels on the carcass, meat quality characteristics, and the intestinal morphology of weaner Large White pigs. Thirty (30) pigs weighing an average of 7.6 $\pm$ 0.54 kg were used for the experiments which were fed different levels of Nano zinc oxide 200, 400 and 600mg/kg and Tylosin 10% as the positive control. After the feeding trial, the pigs were transported to a local abattoir and slaughtered and the carcass characteristics and meat quality parameters (temperature, pH, backfat thickness, drip loss, cooking loss, water holding capacity, and meat colour) were measured and recorded. Cross-sections of the 3 sections of the small intestines were cut and embedded in formalin solution, and later analysed with a microscope for the determination of the villi size. Data obtained were analysed using one way analysis of variance using SAS version 10. Nano zinc oxide supplemented groups had the best comparable results on the slaughter weights compared to the control groups with the least comparable mean values. The positive control group had reduced the backfat thickness and meat pH. The groups fed 200

and 400mg/kg produced darker meat colours while the group fed 600mg/kg of Nano zinc oxide had a brighter meat colour while other meat quality parameters were not significantly (P>0.05) affected by the treatment groups. The supplementation of 600mg/kg had the greatest values of villi height while 400 and 200mg/kg had similar trends. The controls had the least comparable heights of the villi values. Treatment with 600mg/kg of Nano zinc oxide yielded the significant height in the jejunum and ileum villi height than any other treatment, while the treatment of 400mg/kg of Nano zinc oxide had the highest mean value of the jejunum and ileum villi height after the treatment of 600mg/kg of Nano zinc oxide. Treatment with 200mg/kg Nano zinc had the highest mean value of the duodenum across all offered treatments. It can be concluded that the treatment of Nano zinc oxide aided good quality meat thereby giving a good meat colour, a high carcass yield, least pH, and desirable water holding capacity. Also, Nano zinc oxide at doses of 400mg/kg and 600mg/kg can improve the morphology and structure of the small intestine by aiding in the protection against injury and inflammation thereby increasing the height of the villi.

5.2 Introduction

In recent years, there has been a steady rise in demand for animal protein and market awareness (Konkol & Wojnarow ski, 2018). In addition, customers are increasingly opting for safe food, longer shelf life, and high quality freshness. The priority of the pork industry is satisfying market expectations for high-quality pork (Perez, *et al*., 2009) and the fact that consumers prefer better meat increasingly has been demonstrated. It was noted that the customer's decision to buy fresh meat is focused initially on aesthetic attributes such as colour; the lack of marble and purge

(Jonsäll, *et al*., 2000), however, sensory characteristics such as tenderness, juiciness, and flavours of meat were considered to be crucial to consumer buying.

Nanotechnology is widely employed in the food industry to improve the quality of food such as taste, texture, speed of processing, thermal resistance, resilience, and bio-availability (Scrinis & Lyons, 2007; Chaudhry, *et al*., 2008; Sekhon, 2010). Nanotechnology is also being used in food production. Also, the animal processing conditions, protection, and efficiency of their by-products can be enhanced by Nano minerals such as Nano selenium, Nano chromium, Nano Zinc oxide etc. Analysis suggested that these elements and their chelates are best used in the feed industry than inorganic salts (Rajendran, 2013).

Meat is a significant source of calories, nutrients, various minerals, fatty acids, or vitamins in human life, both cultural and religious (Srednicka-Tober, *et al*., 2016). Meat is the predominantly soft muscle. Muscle flesh contains approximately 75% water, 20% carbohydrate, 1-10% fat, and 1% glycogen and 20% protein (Listrat, *et al*., 2016). Flesh consistency must be gradually improved, both in terms of nutrition and organoleptic properties (Schosler & de Boer, 2018), leading to the exponential growth of modern animal agriculture and of market consciousness. Elemike, *et al*. (2019) found out that the livestock sector is looking for new methods of enhancing the overall quality of the feed, and that bio-fortification through the use of Nanotechnology, is one of the most recently produced technologies in the sector (Konkol & Wojnarowski, 2018).

Essentially, zinc is crucial in mammals and birds such as nutrient metabolism, as a component of numerous metalloenzymes, appetite control, regulation of the immune system, oxygen-free

radical scavenging, and in transcription factors, carcass quality etc (Zhao, *et al*., 2014; Liu, *et al*., 2014).

Some of the studies have shown that dietary zinc oxide supplementations at the pharmacology level can protect the intestinal epithelial barrier (Song, *et al*., 2015; Vahjen, *et al*., 2010; Vahjen, *et al*., 2011), improve the diversity of the intestinal microbial community and improve the immune function of post-weaning piglets (Liu, *et al*., 2014). Nano zinc oxide stimulates microbiota and inflammatory response and has the associated capacity to encourage intestinal morphology and inflammation at a high dose of natural zinc oxide in weaning piglets (Long, *et al*., 2017). Physical detachment from the sows in conjunction with immature digestive system/tract and a transition to a strong and complicated diet result in damage to the intestinal structure marked by the lower villi, increased crypt depth, and improved intestinal function (Boudry, *et al*., 2004).

To thwart post-weaning diarrhoea and to boost livestock performance, pharmacological doses of Nano zinc of up to 2000mg/kg have been applied for decades (Poulsen, 1995; Pettigrew, 2006) Li, *et al*. (2012) have found that an improved capacity for in vitro intestinal epithelial regeneration increases in the presence of exogenous zinc oxide and an improvement in intestinal levels insulin-like growth factor (1GF-1 gene) in piglets fed with a high concentration of zinc oxide (Li, *et al*., 2012). The 1GF – 1 gene is a cell growth hormone which may help to cure the intestinal tissue. Studies found that in the first 2 weeks after weaning, the influence of the zinc oxide promoter exists primarily during the time of functional adaptation to the intestinal tract of the piglet (Poulsen, 1995). This research therefore investigated the effect of Nano zinc supplementation of meat quality characteristics.

5.3 Methodology

A total of thirty Large White weaners were raised and supplemented with varying amounts of Nano zinc oxide and were slaughtered after 82 days in a local pig abattoir in Buurhmansdrift, North West Province.

5.3.1 Slaughter procedure

5.3.1.1 Pre-slaughter procedure

Pre-slaughter handling is a major concern in the livestock industry especially the pork industry. Stress applied to livestock before slaughter can lead to undesirable effects on the quality of meat produced from these animals including both PSE (pale, soft and exudative) and DFD (dry, firm and dark). Pre slaughter stress was reduced by keeping the animals cool and with sufficient ventilation and avoiding overcrowding. Before slaughter, animals were starved for 24 hours and had access to water to allow complete bleeding and ease of evisceration.

5.3.1.2 Slaughtering

Slaughtering procedures were carried out with the guidelines of Valdez, *et al*. (2017) with minor alterations. The animals were restrained and electrically stunned to ensure no pain to decrease the stress to acquire the superior meat quality. After stunning, the animals were suspended by their hind limb and moved down a conveyer lane for the slaughter procedures. They were exsanguinated into the thoracic cavity and severance of the carotid artery and jugular vein. This method allows maximum blood removal from the body.

After bleeding, the carcasses were put in a scalding tank for about 4 – 5 minutes in water that is approximately 63 ^{o}C in order to loosen the hair and remove dirt and scuff from the skin. The

carcasses were then placed in a de-hairing machine that uses rubber paddles to remove loosed hair. After cleaning and de-hairing, animals' heads were removed and carcasses were eviscerated and split into two then placed in a cooler.

5.3.1.3 Post-slaughter procedure

The slaughter weights were recorded 24 hours before slaughter followed by the hot carcass, which was taken immediately after slaughter and the cold carcass weight was recorded 24 hours post-slaughter. The pH and threshold parameters were measured and recorded immediately after slaughter and then 24 hours after the slaughter on a breast muscle with a Corning Model 4 pH-temperature meter.

Meat colour readings (L*lightness, a* redness, b*yellowness) were recorded using a colour guide 45/0 BYK-Gardener GmbH reader. Water Holding Capacity (WHC) was determined using the formula: $Water\ holding\ capacity\ (\%) = \frac{initial\ weight - final\ weight\ after\ pressing}{Initial\ weight} \times 100$

Water holding capacity was determined by cutting out a portion of approximately 200g from a thigh muscle then recorded the initial weight of the portion. After recording the initial weight, the sample was then pressed for a period of 120 seconds (2 minutes) then put on a scale to take the readings of the final weight. The water holding capacity was determined using the written formula.

The time difference between initial weight and the final weight measurement was 120 seconds. Whereas cooking loss (CL %) was determined by cutting a portion of approximately 200g from a thigh muscle then cooking it for 30 minutes at a temperature of 180 degrees Celsius then cooking loss was determined using the formula stated as:

$$Cooking\ loss\ (\%) = \frac{weight\ before\ cooking - weight\ after\ cooking}{weight\ before\ cooking} \times 100$$

Drip loss was determined by cutting strips portions of the thigh muscle weighing approximately 10g and suspending them off ground to allow maximum drip in a cold room for 24 hours then using the formula to determine the drip loss.

$$Drip\ loss\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

The intestinal morphology of 30 (6 pigs per treatment) pigs was determined using the methods of (Shen, *et al.*, 2009; Gao, *et al*., 2013) with minor modifications. Briefly, after slaughter, fragments of the small intestine (middle portion of the duodenum, the first portion of the jejunum and distal portion of the ileum) were harvested for morphometric analysis. Approximately 2cm of the transverse tissue were cut and immersed in formalin (10% formalin solution) for 48 hours and later dehydrated and embedded in paraffin wax then sliced to a thickness of 4 micrometres and mounted on to the glass slide (3 glass slides per animal). The glass slides (4 measures per slide) were then subjected to microscopic imaging with a digital camera (Olympus BX5, Olympus Optical Co. LTD. Japan). The height of the villi was then measured using the ZED software to view the images.

Back fat thickness was determined by using a vernier calliper placed across the anterior dorsal fat recorded in millimetres.

5.4 Statistical analysis

The data were analysed using one way ANOVA and Statistical Analysis Software (SAS) version 10. The significant mean among the treatment groups was separated using the Duncan multiple range test in the SAS package. The model is shown below.

Y ijk = μ + DA i + E ijk

Y ijk = dependant variable

U= overall mean

DA i = effect of dietary additive

E ijk = residual error

5.5 Results

Table 7: Meat quality characteristics of weaner pigs fed supplemental levels of Nano zinc oxide

	-ve control	+ve Control	Nano zinc oxide level			SEM
	No additive	Tylosin 10%	200mg	400mg	600mg	
Slaughter weight (kg)	58.00^{b}	58.83^{b}	67.40^{a}	60.67^{ab}	62.75^{ab}	1.11
Hot Carcass Weight (kg)	45.9	43.6	44.12	42.2	42.3	0.95
Cold Carcass Weight(kg)	45.14	41.27	43.8	41.6	41.4	0.99
Dressing Percentage (%)	79.0	74.4	65.9	70.4	67.4	1.92
Back Fat Thickness (cm)	0.76^{ab}	1.1^{a}	0.48^{b}	0.65^{ab}	0.68^{ab}	0.67
pH0	5.79	5.81	5.62	5.70	5.82	5.67

pH24	5.91[ab]	5.73[b]	5.97[ab]	5.88[ab]	5.78[b]	5.80
Meat Colour (L)	44.47[ab]	44.21[ab]	39.48[b]	47.25[a]	45.53[ab]	42.20
Meat Colour (a)	5.68	6.96	4.54	4.58	6.04	4.8
Meat Colour (b)	10.27	10.48	9.53	11.42	11.92	9.92
Water Holding Capacity (g)	11.31	10.94	11.02	11.13	11.30	10.95
Drip Loss(g)	43.46	39.86	44.66	42.16	39.53	39.503
Cooking Loss(g)	30.11	28.18	25.21	26.97	28.47	25.95

[a,b] Means with different superscripts within the same row are significantly differed (P<0.05)

Table 7 shows that the supplemental levels of Nano zinc oxide had a significant (P<0.05) influence on some of the meat quality parameters considered except cooking loss, water holding capacity, drip loss, dressing percentage, carcass weights, and the pH0. Slaughter weights for animals supplemented with 200mg/kg, 400mg/kg, 600mg/kg of Nano zinc oxide were comparably higher than those supplemented with 10% Tylosin, and the negative control.

The meat colour Lightness (L*) ranges (Colour Standards)

Colour	Minimum L* value	Scale
Pale pinkish grey to white	61	1.0
Greyish pink	55	2.0
Reddish pink	49	3.0

Dark reddish pink	43	4.0
Purplish red	37	5.0
Dark purplish red	31	6.0

The meat colour in this study falls within a range of the Red Firm and non- exudative (RFN). The meat in this range is ideal and very desirable and has an appropriate water holding capacity.

Animals offered Tylosin had the highest backfat thickness (BFT) while those on 200mg/kg had the least backfat thickness. Animals fed 400mg/kg, 600mg/kgof Nano zinc oxide and the negative control had a comparable BFT.

The least pH24 was obtained from animals supplemented with 10% Tylosin while the carcass from the animals fed with negative control, 200mg/kg, 400mg/kg, and 600mg/kg of N0061no zinc oxide had similar higher values.

5.6 Results of the villi size in micrometers (µm)

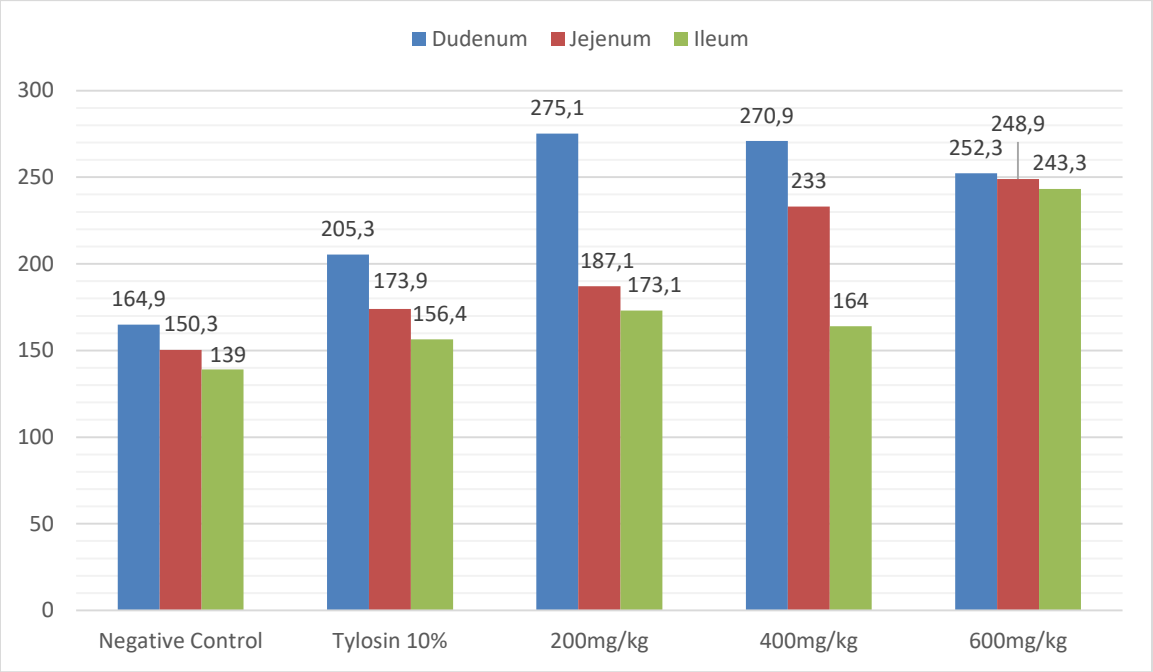
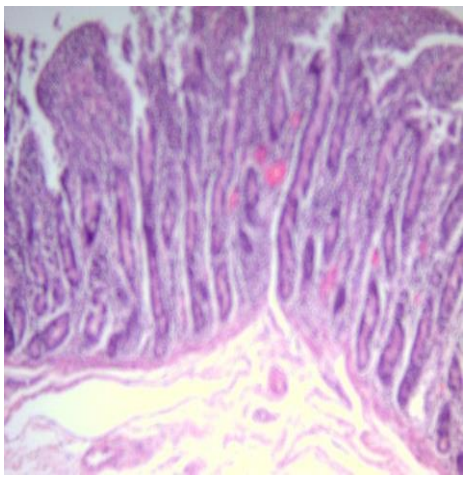
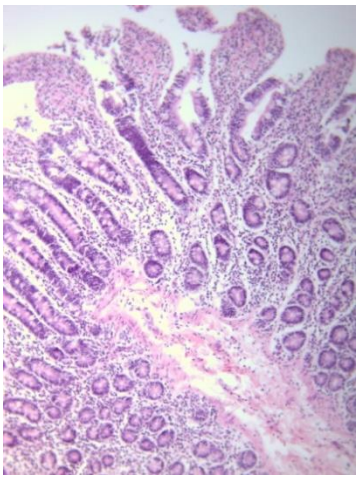


Figure 2 showing the effects of Nano zinc oxide on the intestinal morphology of pigs

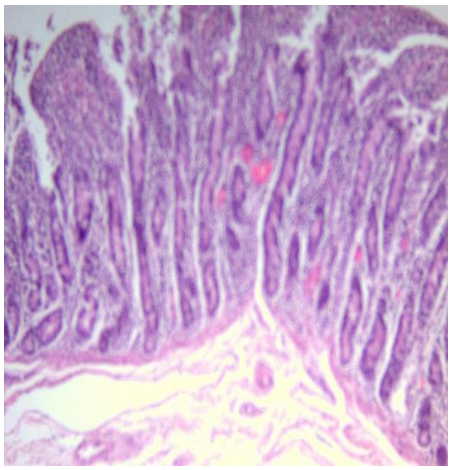
Supplementation of 600mg/kg had a significant effect in the height ($P<0.05$) while 200mg/kg and 400mg/kg had the second-highest comparable height and both the negative and the positive control had the lowest comparable height. The null hypothesis is rejected.

1. Images showing the intestinal morphology of pigs supplemented with 600mg/kg



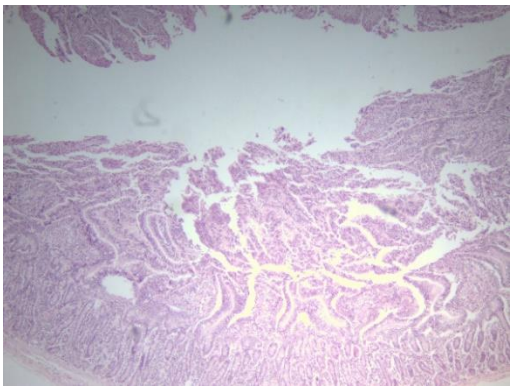


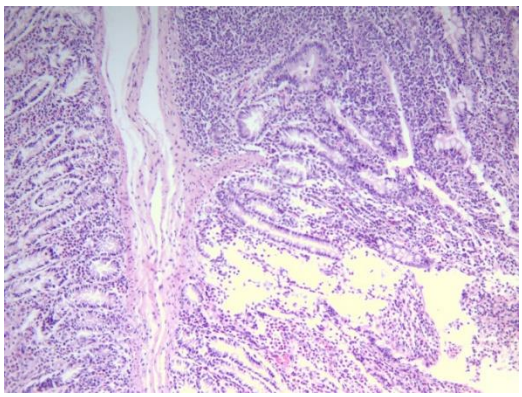
Duodenum Jejenum



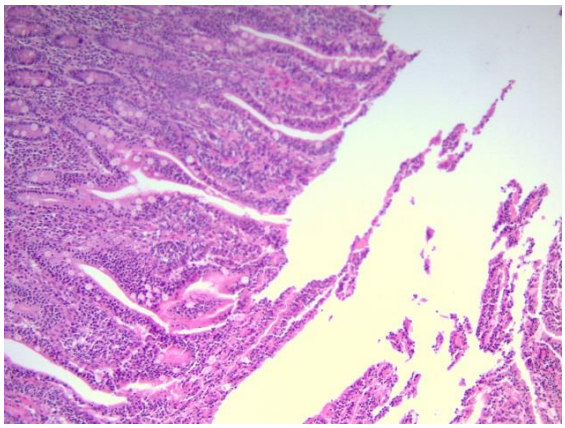
Ileum

2. Images showing the intestinal morphology of pigs supplemented with 400mg/kg



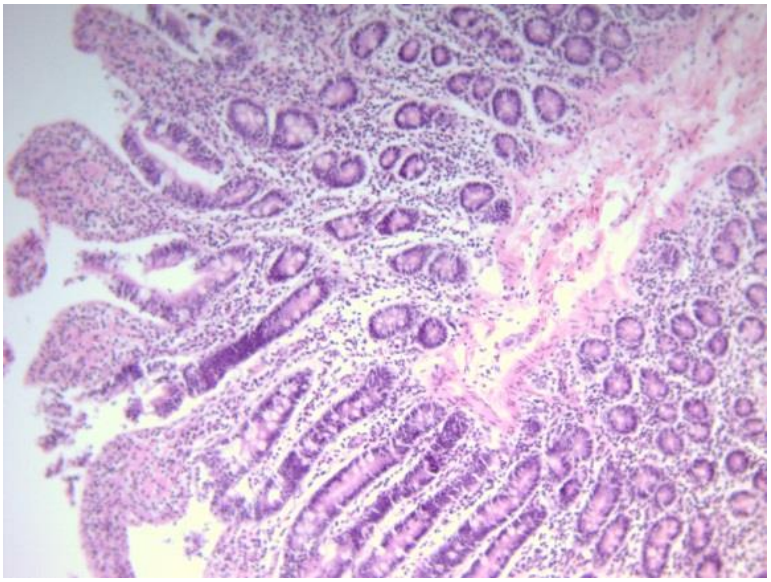


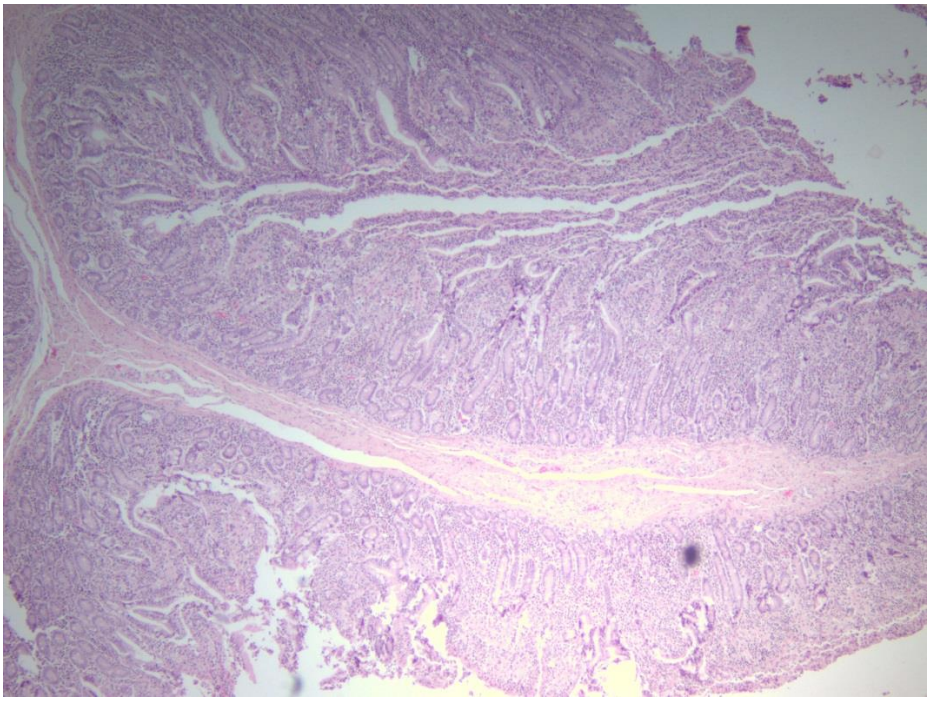
Duodenum Jejenum



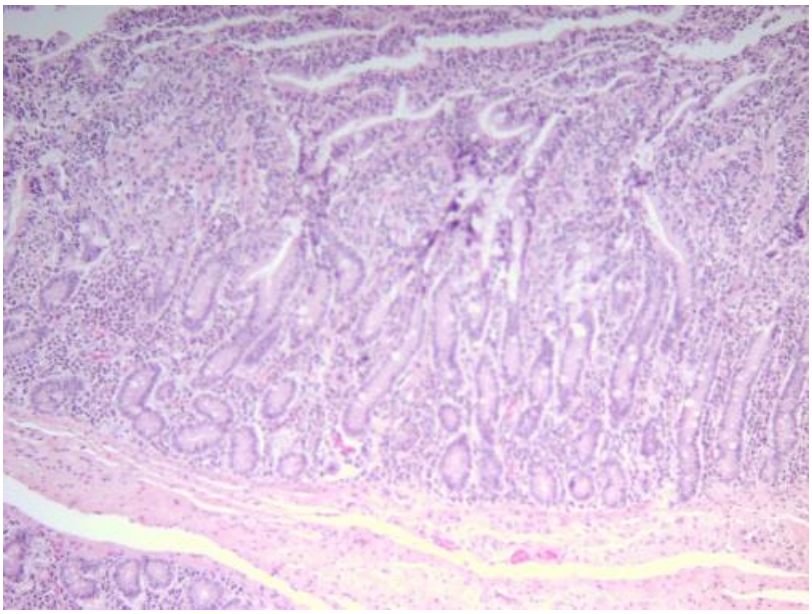
Ileum

3. Image showing the intestinal morphology of the pigs supplemented with 200mg/kg


Duodenum


Ileum

4. Images showing the intestinal morphology of pigs supplemented with Tylosin 10% (positive control)



5.5 Discussion

The colours standards have corresponding L* values where a 1.0 score (pale pinkish grey to white) is equivalent to an L* value of 61, while a 6.0 (dark, purplish-red) has an L* value equivalent to 31 and normal RFN (reddish-pink, firm, and non-exudative) is equivalent to an L* value of 43-49 (NPPC, 1999). All the treatment groups' fall within the normal colour range (44 – 47) apart from the ones supplemented with 200mg/kg of Nano zinc oxide. This can be based on the fact that a lower concentration of nZn, in this case, has been absorbed and used up for tissue growth such that it's not enough for maintaining better meat quality.

Drip loss is associated with the firmness and water holding capacity of the meat. Drip loss is unpleasant, and it can result in excessive cooking losses and drying of meat during cooking (NPPC, 1999). In this study, the non-significantly differed cooking loss and drip loss are below 5% (RFN drip loss is <5%) showing there is no great loss, and this indicated that the meat being moist and having the ability to retain moisture even after cooking.

The pH value, closely associated with the colour, drip loss, and feeding quality characteristics, is the most significant measure of meat quality. When pH values decrease below the optimal range of 5.8 to 6.2 for pork, mild, exudative, or heavy, strong, and droughty meat becomes red. In this analysis, all the pH values were within the optimal range for high-quality pork for the supplemented classes. The development of lactic acid from muscle-glycogen glycolysis after slaughter may be due to any reduced pH values. In Suo, *et al*. (2017), it was observed that, after 14 days of cold storage, the pH of pork containing zinc oxide Nanoparticles filled with films increased to 6.12. The findings of Suo, *et al*. (2017) were consistent with the results of this analysis in pH. In contrast with the negative control group, the Nano zinc substitute had the lowest pH values 24 hours later. The optimal pH values were in the favorable control category (Tylosin 10%) followed by the 600mg / kg Nano zinc oxide supplementation. This indicates that pork pH levels increased considerably during storage by the particulate Nano zinc oxide Nano zinc film coating packaging.

Consistent with the findings of this study, Khah, *et al*. (2015) who found that the proportion of broiler chickens fed Nano-zinc oxide in live body weight and carcass weight improved substantially. They also documented increased breast and thigh weight in birds that were fed a diet consisting of 60mg/kg or 90mg/kg Nano zinc. In line with this present study, Lina, *et al*. (2009) were also of the opinion that zinc Nanoparticles can increase production performance as

well as the dressing percentage of broiler chickens within 42 days of feeding 40mg/kg Nano zinc oxide in the diet. In a related study by Xu, *et al.* (2017), pigs supplemented with chromium methionine and different sources of zinc it was discovered that the hot carcass weight, dressing percentage, and loin muscle area in pigs fed with diets supplemented with both Chromium methionine and ZnA were increased compared to those supplemented with $ZnSO_4$ only. Loin meat colour and drip loss were not affected by the treatment, which is in accordance with the results of this study. The meat colour in this study falls within a range of the Red Firm and non-exudative (RFN) (Reddish pink to dark reddish pink with L*value of between 49 and 37 and 4.0 and 3.0. The meat in this range is ideal and very desirable and has an appropriate water holding capacity

Early weaning of the stress symptom usually contributes to a reduction in villus height and intestinal disorder in piglets. The tiny gut epithelium functions as a deterrent to noxious antigens and bacteria and also helps to digest and consume nutrients (Wijtten, *et al*., 2011). Impaired intestinal epithelial function disrupts immune homeostasis and increases inflammation, disturbing the intestinal barrier function and the entrance of allergenic compounds from the gut into the body resulting in immunologic responses and an increased chance of contracting infections (Wijtten, *et al*., 2011). In a study by Wang, *et al*. (2018), it was revealed that the supplementation of 800mg/kg Nano zinc oxide had improved the intestinal morphology which is in agreement with the results of this study that proved that Nano zinc oxide at doses of 600mg/kg can give better results as compared to lesser doses. Results of this study indicated that doses of 600mg/kg and 400mg/kg protected the small intestinal morphology by increasing the height of the villi, which was consistent with the results reported by Long, *et al.* (2017) who reported that the supplementation of 500mg/kg of Nano zinc protected against injury of the small intestine.

Wang, *et al*, (2018) reported that zinc oxide and colistin sulphate did not affect the duodenal villi width but significantly increased duodenal villi length and surface area. Analysis of the ileal mucosa morphology revealed that dietary Nano zinc oxide and colistin sulphate + zinc oxide significantly increased the villus length, width, and surface area ($P<0.05$) which is in agreement with the observation of this study. In a study by Hu, *et al*. (2014) where he was experimenting whether zinc oxide supplementation could alleviate weanling- induced intestinal injury through Toll-like Receptors (TLR) and NOD-like Receptor (NLR) signalling pathways he found out that supplementing of zinc oxide increased the villus height: crypt depth ratio at the jejunal mucosa is consistent with the previous reports by Song, *et al*, (2015) who were evaluating the effects of Diosmectite zinc oxide composite on the internal barrier function, modulates expression of proinflammatory cytokines and tight junction protein in early-weaned pigs and Hu, *et al*. (2014) who evaluated the effects of montmorillonite-zinc oxide hybrid on performance, diarrhea, intestinal, permeability and morphology of weaning piglets which are also consistent with the results of this current study and further buttresses the positive effects of the supplementation of Nano zinc oxide.

5.6 Conclusion

The treatments influenced most of the meat quality parameters. The supplementation of 200mg/kg of Nano zinc oxide on pigs gives the pigs with the least back fat thickness while the supplementation of Tylosin 100mg/kg gives pigs with much backfat thickness, therefore the null hypothesis is rejected. All the treatments had the best meat colour but the supplementation of 400mg/kg gives the meat a very bright and reddish pinkish colour that is desirable. The null hypothesis is also rejected. Drip loss and pH were all normal but Tylosin gives the least pH and consequently less drip loss, therefore the null hypothesis on meat colour is accepted due to the

insignificant differences in the means. Overall, the supplementation of 400mg/kg of Nano zinc oxide is ultimate in the production of pigs with superior meat quality characteristics. The supplementation of Nano zinc oxide at doses of 400mg/kg and 600mg/kg can improve the morphology and structure of the small intestine by aiding in the protection against injury and inflammation thereby increasing the height of the villi. The null hypothesis is rejected.

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CHAPTER 6

General conclusions and recommendations

This study was carried out to evaluate the effects of Nano zinc oxide supplemented at 200mg/kg, 400mg/kg, 600mg/kg, and Tylosin 10% as a positive control and the negative group (no additive) on the immune response, the physiological characteristics and the growth performance of Large White weaners. With all the data collected and analysed, it can be concluded that the supplementation of Nano zinc oxide at 200mg/kg improves the growth performance of pigs from the weaner stage until the finishing stage.

To understand the effects of adding Nano zinc oxide to the basal diets of pigs; further research is required to elaborate on the functional mechanism; absorption, translocation, and interaction with other elements or nutrients.

The inclusion of zinc at the dose of 600mg/kg is highly recommended in the feeding of pigs for improvement of growth, immune response, and good meat quality and carcass yield.