

# **Response in fertility of Windsnyer boars to increasing $\alpha$ -tocopherol supplementation**

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**By**

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**Declaration**

I, **Bovula Ntombizodwa**, vow that this dissertation has not been submitted to any other University other than the University of KwaZulu-Natal and that it is my original work conducted under the supervision of Prof M. Chimonyo and Prof T.L. Nendambale. However, works of other researchers and authors, which served as sources of information, were duly acknowledged.

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## Abstract

The broad objective of the study was to determine the response of  $\alpha$ -tocopherol inclusion level supplementation on fertility of Windsnyer boars. The specific objectives of the study were to determine the response of  $\alpha$ -tocopherol inclusion level on growth performance, testicular development and epididymal spermatozoa characteristic of Windsnyer boar boars. Twenty Windsnyer boars aged 3 months old with an average body weight of 19 kg were selected. Each pig was housed individually in a 1.54 x 0.8 m pen in environmentally controlled house with the temperature ranging from 22 to 25<sup>0</sup>C. Pigs were kept until they reached an average weight of 57 kg. Five boars were randomly assigned to each diet containing 0, 40, 70 and 90 IU levels of  $\alpha$ -tocopherol. The boars were fed 1.5 kg per day and water was provided *ad libitum* through drinking nipples. The feed intake and growth performance was estimated. At age of 21 months, the boars were slaughtered and testicular development was measured. Semen was harvested from the epididymis following euthanization and assessed using the computer aided sperm analysis software for spermatozoa characteristics. Polynomial regression analysis was used to determine the relationships between  $\alpha$ -tocopherol inclusion level and growth performance, testicular development and spermatozoa characteristics.

Supplementation of  $\alpha$ -tocopherol inclusion levels had a quadratic relationship to body weight of Windsnyer boars. There was quadratic relationship between ADG and  $\alpha$ -tocopherol levels ( $y = -0.0083x^2 + 0.7786x - 0.08$ ;  $P < 0.05$ ). There was no relationship between  $\alpha$ -tocopherol inclusion and testicular development. There was, however, a tendency of seminiferous tubule to be influenced by  $\alpha$ -tocopherol inclusion. There was no relationship between  $\alpha$ -tocopherol and volume, pH and spermatozoa motility. There was a quadratic increase in spermatozoa concentration with

increasing levels of  $\alpha$ -tocopherol. As the  $\alpha$ -tocopherol levels increased, straight line velocity (VSL) increased in a quadratic manner ( $P < 0.05$ ). The equation was  $y = 0.0068x^2 + 0.7679x + 21.983$ . There was also a quadratic increase ( $P < 0.05$ ) in live spermatozoa percentage, as  $\alpha$ -tocopherol inclusion increase. The equation was:  $y = 0.004x^2 + 0.395x + 79.4$ . The proportion of spermatozoa with abnormal head decreased quadratically ( $P < 0.05$ ) with  $\alpha$ -tocopherol inclusion level. The equation was:  $(y = 0.0011x^2 - 0.1064x + 3.5917)$ . In conclusion,  $\alpha$ -tocopherol quadratic relationship body weight, average daily gain, spermatozoa concentration, live, dead and abnormal head spermatozoa. VSL was linear had linear relationship with  $\alpha$ -tocopherol inclusion levels of Windsnyer boars.

**Key words:** computer aided sperm analysis software, epididymal spermatozoa, growth performance, testicular development, live spermatozoa percentage, semen volume, semen pH, spermatozoa abnormality, spermatozoa concentration, spermatozoa motility.

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This dissertation is dedication to the Nyamezele and Bovula's family, particularly my late parents

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To my daughter Seneziwe Sesona Philasande for growing up without me.

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## CHAPTER 1: General introduction

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### 1.1 Background

Windsnyer pigs are native in Southern Africa. They are found mostly in found in parts of Mozambique, Northern Zimbabwe and eastern parts of South Africa (Halimani *et al.*, 2010). They play a fundamental role in livelihood of resource-limited households. They provide nutritious pork and high levels subcutaneous fat is used as lard for cooking purposes. The Windsnyer pig genotype is adapted to climatic change-induced harsh conditions (Ramsay *et al.*, 1994). Windsnyer pigs are early maturing, but grow slower than the exotic breeds (Madzimure, 2011). The population of Windsnyer pigs has dropped markedly and they are danger of extinction (Halimani *et al.*, 2010).

Indiscriminate uncontrolled crossbreeding, political instability and globalisation are among factors are leading to lack of interest in utilizing Windsnyer pigs. In addition, pig production organizations in Southern Africa are in favour of imported breeds and are solely promoting them to be used in smallholder production system. The outbreak of Classical Swine Fever also led to extensive culling of pigs, particularly those in rural production systems (Halimani *et al.*, 2010). The Windsnyer pigs are shuns due to their compact body and slow growth rate (Chimonyo *et al.*, 2011). However, imported breeds struggle to survive and reproduce efficiently in smallholder production conditions such as poor planes of nutrition, high diseases prevalence and harsh environments (Chimonyo *et al.*, 2011). This necessitates a need to conserve Windsnyer pigs for reproductive purposes. South Africa is arguably the only country in Southern African that has an existing policy about

conservation of indigenous pigs. For example, the Agricultural Research Council of South Africa is keeping large number of Windsnyer pigs for breeding and research.

Thus, these pigs are not considered for pork production because of their short carcasses, which cannot be simply prepared into specialised pork portions (Chimonyo *et al.*, 2010). Windsnyer pigs have small frame size, with mature weight of about 100 kg. They have low maintenance and growth nutrient requirements. They are predominantly black with long nose and have a stocky body. Imported breeds such as Large White pigs have superior fertility and growth rates (Ncube *et al.*, 2003). They, however, have potential to improve food security, reduce poverty and improve the livelihood of resource-limited farmers. In addition, they are viewed as a suitable breed for resource-limited farmers because of their tolerance to various disease and adaptability to adverse condition.

It is evident that there is potential for the improvement of the growth and fertility of the Windsnyer pigs. To maintain a high reproductive performance, breeding animals should reach puberty at an appropriate age or body weight. The reproductive performance of boars is usually monitored by analysing spermatozoa quality. Vitamin E (particularly  $\alpha$ -tocopherol) is essential for integrity and optimum function of reproductive, muscular, circulatory, nervous and immune systems (McDowell, 2000). Vitamin E reacts as membrane bound antioxidant, trapping lipid peroxyl free radicals produced from unsaturated fatty acids. Boar spermatozoa are sensitive to peroxidative damage due to high content of unsaturated fatty acids. Little, if any, is known about the effect of  $\alpha$ -tocopherol supplementation on spermatozoa quality of Windsnyer boars.

## **1.2 Justification**

The information to be generated from study assists farmers in identifying appropriate level of  $\alpha$ -tocopherol to improve fertility of Windsnyer boars. It also helps in strengthening the need to conserved Windsnyer pigs. *Ex-situ* and *in-situ* to become an important source of genes pool with characteristics of economic importance for the future to meet the ever-changing consumer needs and climate change. Global change temperature has an effect on pig production (Scholtz, 2009). Conservation programmes and appropriate policies are required for Windsnyer pig genetics because of their resistance to diseases and good adaptability to low input production system (Halimani *et al.*, 2012), can assist farmers to survive future environmental shocks. Considering that the working boar is expected to be healthy individual able to produce large number fertile active spermatozoa and to have good general longevity, antioxidants ( $\alpha$ -tocopherol) must supplemented to breeding boars so that can improve reproductive performance. Governments need to take active role in promoting the Windsnyer pigs in terms of service delivery for their contribution to the conservation (FAO, 2007).

## **1.3 Objectives**

The broad objective of the study was to determine the response in fertility of Windsnyer boars to increasing  $\alpha$ -tocopherol supplementation. The specific objectives were to:

1. Determine the response of growth performance and testicular development of Windsnyer boar to  $\alpha$ -tocopherol supplementation; and
2. Assess the response of Windsnyer spermatozoa characteristics to  $\alpha$ -tocopherol inclusion.

## 1.4 Hypotheses

The hypotheses to be tested were that

1. Alpha-tocopherol inclusion influences growth performance and testicular development rate of Windsnyer boar; and
2. Alpha-tocopherol inclusion influences spermatozoa characteristics Windsnyer boars.

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### 2.1 Introduction

The pig population is growing fast because of the quick turnover of capital and increasing demand. On a worldwide basis, more meat is produced from pigs than from any other domestic species. It is estimated that over 65 % of the world's pig population is in the poorer developing regions, representing over 500 million pigs (Holness *et al.*, 2005). In Africa, pork production is around 565 000 tons per annum (McGlone and Pond, 2003). Large population of Windsnyer pigs in the communal area shows the potential contribution of these pigs to meat production and livelihoods of rural communities. Hafez, (1974) suggested the possibility that other vitamins supplementation may improve fertility rate of boars. McDowell *et al.* (1996) highlighted that vitamin E ( $\alpha$ -tocopherol) is essential for fertility as well as growth, prevention of diseases and the integrity of tissues. The review highlights the importance of Windsnyer pigs and challenges of Windsnyer pig production. It also discussed the measures of semen quality, factors affecting boar fertility and role of  $\alpha$ -tocopherol.

### 2.2 Importance of Windsnyer pigs

The name Windsnyer (wind-cutter) is derived from its shape as it is narrow-bodied and long nosed (Halimani *et al.*, 2010). Like the Kolbroek, Windsnyer is hardy and scavenges for its food. The Windsnyer pig is also able to survive and reproduce under food shortages. They are usually of modest size with adults reaching a maximum weight of 100 kg but rarely more than 60 kg at 12 months of age, even under the best rearing conditions. The pigs are sexually early maturing. Females may show first oestrus as early as three months of age. Windsnyer pig population was estimated at 19 million in 2001 (Pathiraja, 1987).

Windsnyer pig breeds play an important role in sustaining rural livelihoods and utilizing marginal ecological areas. These pigs provide a wide range of products which include pork and lard. They also yield non-monetary benefits such as manure that is vital to sustain intensive crop cultivation by being a component of indigenous rituals and social exchange systems. Indigenous pigs are largely related to their hardiness and adaptation to survive under smallholder production environments where resources are limited. These pigs are adapted to the low and medium input environmental systems and attain puberty at 150 days of age (Holness *et al.*, 2005).

Windsnyer pigs are tolerant to warm climates, and are often found near rural villages where they are reared at free range or backyard. They have a potential to increase food security, reduce poverty and improve the livelihood of resource-limited farmers. They are suitable for breeding purposes for resource-limited farmers because of their tolerance to various diseases and adaptability to adverse conditions (Madzimore, 2011). Windsnyer pigs survive under conditions with inefficient breeding management, insufficient veterinary care, and inadequate feeds and feed management (Chimonyo *et al.*, 2010). They can survive on cheap, high-fibre diets and can convert feed with low nutrient content very efficiently (Ndindana *et al.*, 2002). Windsnyer pigs produce tasty meat, have excellent foraging ability and good mothering ability (Chimonyo *et al.*, 2005). These traits of economic importance that make this breed favourable to communal production system and determine the potential profit for the famers.

### **2.3 Challenges to Windsnyer pig production**

There is also a limited number of Windsnyer pigs, leading to the use of less appropriate exotic breeds in restocking programmes. The national systems of grading carcass of pigs overlook the Windsnyer breeds due to their black hair follicles and thicker back fat. Therefore, it discourages the use of indigenous pigs. This lead to compound lack of formal markets for Windsnyer pigs (FAO, 2004). Halimani *et al.* (2012) reported that exotic breeds influence farmers to abandon the use of slow-growing Windsnyer breeds. Windsnyer breed are not well regarded due to their slow growth, low feed conversion efficiency (Chimonyo *et al.*, 2005), low fertility and high fat deposition (Ncube *et al.*, 2003).

The focus on increasing pig production has been on the introduction of imported pigs that have been selected for production traits such as growth rate (Drucker *et al.*, 2000). The introduction of imported pigs has led to genetic erosion of Windsnyer pig and the destabilisation of the traditional livestock production systems (Chimonyo *et al.*, 2005). Windsnyer pigs are often crossed with imported boars to take advantage of heterosis (Madzimore, 2011). Windsnyer pigs are under threat from outbreak of diseases. For example, in 2005, there was a classical swine fever disease outbreak in the Eastern Cape Province of South Africa, which led to culling of more than 335 000 pigs (NAFU, 2007; Halimani *et al.*, 2010). Poorly planned conservation practices can lead to genetic erosion of local pigs. This could be due to intense inbreeding in the small populations, inadequate storage of genetic material and ex-situ conservation (Madzimore, 2011). The major challenges to pig production using Windsnyer pigs include low levels of management, high propensity for fat deposition, lack of formal marketing channels and low fertility.

### **2.3.1 Low level of management**

Windsnyer pigs require less or no labour to survive since there is minimum management. Windsnyer pig production is a cheap resource-limited feed supply by going for alternatives such as rotten maize, hominy chops, coarse maize meal, kitchen waste, vegetables, pumpkins, groundnut shells, fruits, grasses and brewers waste (Scherf, 1990). These feeds are high in fibre but low in protein, which make them appropriate for slow-growing pigs which efficiently converts poor quality (Kanengoni *et al.*, 2002; Chimonyo *et al.*, 2005). These pigs are kept under free range conditions, rely on low inputs and technology. The pigs are exposed to high risks of predation as they scavenge and may consume intestinal parasite eggs during foraging and muddy yards during the rainy season thereby increasing intestinal worm burdens. Their survival under these conditions would testify to their disease tolerance. They have greater tolerance to nematodes such as *Ascaris suum* than imported breeds (Zanga *et al.*, 2003). Capital for pig herd health programmes for disease control and prevention and recorded-keeping being limited. Access to capital is major limiting factor, especially in view of the high interest rates and long-term nature of investing in livestock (Lekule and Kyvgaard, 2003). See (1996; 2000) showed that reproductive performance of boars and sows which were not receiving adequate nutrients was lower than those that received a balanced diet. There is lack labour, feed and capital leads lack of proper weaning and breeding strategy, to the extent that this results in several undesirable consequences such as slow growth rate, reduced number of matings per week, reduced litter size at birth. Most importantly, these challenges reduce the number of piglets weaned/ sow/ year (Fasina, 2012).

### **2.3.2 High fat deposition**

The Windsnyer pigs are susceptible to depositing a lot of fat due to their inherent slow growth. Excess energy is partitioned towards fat deposition and thus a low-energy diet will ensure that the pig's energy requirement is met. Windsnyer pigs have poor body conformation and low dressing weights. The cold dressed weights are about 65 kg in Large White pigs and 37 kg for the Windsnyer pig at about 20 weeks of age. Local pigs fail to meet the grade for pork, or achieve good grades (Kanengoni *et al.*, 2004). The Windsnyer pig can deposit up to 30 mm of fat subcutaneously at the P2 and K7.5 mm position compared to 11 mm for the Large White pig (Kanengoni *et al.*, 2004). The fat deposit on Windsnyer carcass could be easily trimmed off to yield a leaner carcass and the fat used for other functions such as cooking (Chimonyo *et al.*, 2005). The Windsnyer pig has a smaller eye muscle compared to the imported pig (Kanengoni *et al.*, 2004). The Windsnyer breed is discriminated against the market because of their short carcasses, which cannot be easily cut into specialised pork portions (Nicolas, 1999; Chimonyo *et al.*, 2010). There is, therefore, a need to determine the opportunity of using South African Windsnyer pig breeds to produce pork for fresh consumption.

### **2.3.3 Lack of formal marketing channels**

The national systems of grading carcasses of pigs penalize the local breeds due to their black hair follicles and thicker backfat and, therefore, discourage the use of local pigs. This has also led to a compounded lack of formal market for local pigs (FAO, 2004). Lack of economic values for local pigs has led to the lack of appreciation of their economic roles. The social and cultural values of traits for local pigs are not captured in the market place (Roessler *et al.*, 2008).

#### **2.3.4 Low fertility**

Reproductive performance of Windsnyer pigs in Southern Africa is low. Interestingly, the pigs reach maturity early compared to Large White (Holness and Smith, 1973). Gilts show first oestrus as early as three months of age or when reach body weights of about 21 kg. The Windsnyer pigs has favourable litter size of 7, which is over 90 % lower than imported pigs (Chimonyo *et al.*, 2008). It is not clear whether improved feeding and management of the Windsnyer pigs could increase the number of litter at farrowing (Holness and Smith, 1973). The fertility at weaning of imported genotypes is equivalent to about two and half times that of Windsnyer sows (Agricultural Research Council, 2010).

To improve fertility of pigs, it is crucial that the measures of fertility are clearly understood.

#### **2.4 Measures of boar fertility**

Boar have a profound influence on the proportion of females that are mated, proportion of mated sows that conceive and farrow and on the number of piglets per litter. Boars associated with high fertility and large litters consistently produce ejaculates that contain sufficient numbers of spermatozoa (Koketsu *et al.*, 1999; Willenburg *et al.*, 2003).

The detection of difference in fertility between males is of importance to breeders and producers. Madsen *et al.* (1992) highlighted that the primary determinant of boar fertility is its libido. Superior boars are also assessed by utilising the motility and morphology of spermatozoa, conception rate and litter size (Broekhuijse *et al.*, 2012). Subfertility or sterility in boars that are ultimately attributable to the quantity and quality of the semen (Bonet, 1990). In general, measures of fertility

in boars are categorised into testicular development, growth performance of boars and spermatozoa characteristics.

#### **2.4.1 Testicular development**

Testicular histology is used to determine daily spermatozoa production in boars (Kęstutis *et al.*, 2011). Changes in the structure and position of testes have a direct role in spermatozoa production disorders or qualitative parameters. Acquired testicular histology has been determined much more frequently than congenital or inherited disorders. Kęstutis *et al.* (2011) reported that inherited or congenital hypoplasia of the seminiferous tubules and degeneration are the two most common lesions which affect disturbance in spermatogenesis.

The testis is made up of seminiferous tubules where the spermatozoa are formed. The tubules are two-ended convoluted loops with both ends opening into the rete testis, through which spermatozoa pass on their way to the excurrent duct system. In pigs, seminiferous tubule diameters range from 160 to 350  $\mu\text{m}$  (Walker *et al.*, 2004).

##### ***2.4.4.1 Seminiferous tubules***

Seminiferous tubules are divided into three compartments, basal, adluminal and luminal compartment. The basal compartment consists of basal cell membrane, spermatogonia and Sertoli cell. The adluminal compartment, in which differentiation of spermatocyte occurs, consists of primary and secondary spermatocytes, round spermatids and elongate spermatids. The luminal compartment consists of spermatozoa that were released from the Sertoli cells. The basal and

adluminal compartment are separated by intercellular structures between adjacent Sertoli cells, which forms a continuous lock, so called the blood testis-barrier (Ritzen, 1983).

#### ***2.4.4.2 Spermatogonia***

Situated at the base of the seminiferous epithelium, the spermatogonia act as stem cell. The spermatogonia are proliferative and transform into differentiating spermatogonia are proliferative and transform into differentiating spermatogonia and simultaneously renew their population by mitotic division. The replication of spermatogonia are dependent on the expression of c-kit protein synthesis by Sertoli cells (de Krester *et al.*, 1998). There are different class of spermatogonia which is type A and type B. The differences among these types of spermatogonia are based on structure and morphology, using either light or electron microscopy.

#### ***2.4.4.3 Sertoli cells***

Sertoli cells are fibroblast-like cells, which provides mechanical support for the germ cell during spermatogenesis. Sertoli cells acts as functional and structural bridges linking the blood-lymphatic intertubular space to protect seminiferous luminal compartment where the spermatozoa are transported to the epididymis (Kierszenbaum, 1994). There are several chemical messengers between Sertoli cells and germ cells. Some properties of Sertoli cell that affect germ cell include tight junction between the Sertoli cells, which prevents all macromolecules and some electrolytes from circulation to germ cells. All hormones exert their effects on germ cells through Sertoli cells. They secrete large quantities of lactate, the substrate for glycolysis used by germ cells for energy metabolism. Sertoli cell activity is regulated by testosterone from Leydig cells and follicle-

stimulating hormone (FSH) secreted from the anterior pituitary gland. Sertoli cell produces androgen-binding protein (ABP), insulin-like growth factor I (IGF-I) (Dym, 1994).

#### ***2.4.4.4 Leydig cells***

Leydig cells are located in the inter-tubular space, produce and secrete androgens, mainly testosterone, in response to stimulation by luteinizing hormone (LH), released from the anterior pituitary gland (Sharpe, 1994). The main substrate for the synthesis of steroid hormones in Leydig cells is cholesterol. There are three enzymes, which are cytochrome P- 450<sub>sc</sub> (cholesterol side chain cleavage), adrenodoxin reductase, involved in the conversion of cholesterol to pregnenolone. Luteinising (LH) is required for maintenance of Leydig cell-specific functions and is the main controlling factor for testosterone secretion from Leydig cells (Saez, 1994). Testosterone production by Leydig cells is dependent on LH stimulation from the anterior pituitary gland and follicle stimulating hormone (FSH) stimulation through the Sertoli cells, thereby suggested a strong endocrine regulation (Saez, 1994).

#### ***2.4.4.5 Spermatogenesis***

Spermatogenesis is the sequence of cellular divisions and developmental changes that occur within the seminiferous tubules of the testes (Eddy and O'Brien, 1994). The spermatogenetic process is comprised of the two major processes which is spermatocytogenesis that contains two processes. Mitotic process of stem cells to form spermatocytes and meiosis to reduce the number of chromosomes to form spermatids. The second stage is known as spermiogenesis, which is the transformation of spermatids in regard to metamorphic changes. Spermatocytogenesis and spermiogenesis are closely associated with Sertoli cells, the nurse cells for spermatozoon inside

the seminiferous tubules. The Sertoli cells contribute to the blood testis-barrier and supply the nutrients needed for spermatogenesis.

#### **2.4.4.6 Libido**

Libido or sexual desire, exemplified by reaction time, is an important aspect of male reproductive function (Umesiobi and Iloeje, 1999). It may be impaired by mismanagement of young boars during service (Hafez and Hafez, 2000). Boars with high testosterone tend to have high levels of libido (Flowers, 2008). Sexual behaviour has evolved to ultimately result in the deposition of sufficient viable spermatozoa in the reproductive tract of the female at the optimum time. Low levels of sexual behaviour result from either low sexual motivation or poor mating competency. Male sexual behaviour is obviously necessary to achieve impregnation in natural mating systems. The level of male sexual behaviour may affect reproductive performance (Hemsworth and Tilbrook, 2007).

#### **2.4.2 Growth performance**

Growth performance defines the process whereby pigs increase in physical size. Maturity is driven by animal's current size or mass, age and nutrient supply. Growth performance is measured by pig's average daily gain (ADG), feed intake and feed conversion rates (Whittemore and Kyriazakis, 2006). Too rapid growth rates are not ideal for fertility. Slow growth rate is an inherent setback in the use of Windsnyer pigs for commercial production. Nevertheless, the purported slow growth syndrome attributed to the indigenous pigs can be an advantage production systems.

#### **2.4.2.1 Average daily gain**

Low piglet weight at weaning implies a loss of income for the farmer and might influence the welfare of the affected animals. The ADG of piglets from birth to weaning. Body weight gain increased with level of feeding. Heritability of pre-weaning ADG of 0.21, 0.08 was estimated by Grandinson, (2003). The ADG of pigs raised under extensive system is 0.639 kg and the pigs that are in intensive production system were 0.725 kg (Enfalt *et al.*, 1997). Pig diets are made up of rice bran, coarsely ground maize and weeds that are available in fallow land. These diets are low in crude protein resulting in poor pig performance. This results in slow growth and low performance and productivity of the pigs (Hansen, 1997).

Windsnyer pigs exhibit relatively low growth rate 250 g/day compared to 960 g/day for Large White pigs under restricted feeding (Kanengoni *et al.*, 2004). These pigs show a remarkable peak in growth between 12 and 14 weeks post-weaning. Associated with early maturation, resulting in early deposition than the fast-growing imported pigs (Chimonyo *et al.*, 2005). Mukota pigs reach slaughter weight of 35 to 40 kg at six months of age, in contrast to Large White pigs which reach slaughter weight of 100 kg at similar age (Chimonyo *et al.*, 2005). Hence, there is a need to evaluate the response of vitamin E on growth performance of Windsnyer pigs. Boars are more efficient compared to gilts and an increase in slaughter weight of boars is associated with an increase in average daily (ADG) and gain: feed ratio (Barton-Gade, 1987). Boars with the average testis volume of 1000 cm<sup>3</sup> showed a higher ADG, superior performance index, as well as higher muscle depth and higher body mass at the end of the performance test (840.8 g/day and 84.8 kg, respectively). Boars with small testis size have lower average performance characteristics (daily

gain, performance index, eye muscle depth and body mass: 786 g/day and 80.8 kg, respectively) (Janyk, 2004).

#### ***2.4.2.2 Feed intake***

Feed intake is the key to developing diet specifications, attaining growth rates and has huge impact on efficiency of production. Residual feed intake is merely the difference between the expected intake of the pigs and what they consume. The rate feed intake is generally associated with the pigs attempts to maintain dry energy intake (Adesehinwa, 2008). Feed intake of pigs determines nutrient intake levels and thus has a great impact on efficiency of pork production. Local pigs can convert feed with low nutrient content efficiently, enabling them to survive on fibrous feedstuffs, such as maize cobs and cereal by-products of brewing. There is a need to assess the influence of  $\alpha$ -tocopherol, which is expected to increase boar fertility, influence feed conversion ratio of Windsnyer boars.

#### ***2.4.2.3 Feed conversion efficiency***

Feed conversion efficiency (FCE) indicates the quantity of feed required to lay down a unit of body tissue. Carcass feed conversion efficiency is also used to indicate the quantity of feed required to lay down unit of carcass tissue. The higher the quality of the feed the better animal will convert it to body tissue. The more palatable the feed the high the intake. Feed conversion efficiency is of growth performance traits and can be defined as the efficiency with which feed consumed is converted to muscle. Feed costs accounts for much of total production costs, about 60 to 70 % (Lasley, 1993).

The feed conversion rate is expressed as the amount of feed that is required to gain 1 kg of body weight (Booth, 1995). Feed conversion is about 60 % correlated with growth rate. The actual feed conversion rate depends on environmental conditions. Feed conversion efficiency of pigs that raised under extensive production system is 2.91. Feed conversion into muscle in Windsnyer pigs is low since most of the dietary nutrients are converted into fats. The relationship between feed conversion efficiency and fertility in pigs is not clearly understood.

Besides understanding growth performance of pigs, herd fertility is improved if spermatozoa characteristics are also clearly determined. It is argued that the most important traits are spermatozoa concentrations and motility.

### **2.4.3 Boar spermatozoa characteristics**

Boar semen is a suspension of spermatozoa cells and secretions from the boar reproduction tract, including the accessory glands. The fluid portion of this suspension is known as seminal plasma and it helps to carry and protect the spermatozoa. Boar semen is characterised by a large volume (average 225 ml gel free portion, but ranging from 150-500 ml) (NRC, 1998). The main components of the spermatozoa are the head, which contains the DNA that is transmitted to progeny, a midpiece that is involved in energy production for the tail piece and the tail that allows the spermatozoa to swim. The length of individual parts of the spermatozoa cell varies from different species. The sperm flagellum is long and thin in most animal species. For motility, the flagellum use ATP that is generated from mitochondria in the midpiece of sperm. In all animal species, the colour is creamy white and the temperature is approximately 37.5<sup>0</sup>C.

#### ***2.4.3.1 Semen volume***

Visual evaluation of the opacity of the ejaculate gives an idea on the spermatozoa concentration (Masenya, 2012). Boar ejaculates vary between 150 and 300ml (Garner and Hafez, 1986). The volume is subject to considerable variation because of individual characteristics and environmental conditions.

Ejaculation of boars occurs in three phases namely, (i) pre-spermatozoa fraction consist of 5 and 15 ml of a colourless water fluid containing few spermatozoa, (ii) a spermatozoa rich fraction which is milky white making up about 15 % of the ejaculate volume containing up to 90 % of the spermatozoa could withstand handling procedures better than those contained in the latter part of a fractionated ejaculate (Masenya, 2012) and (iii) post sperm fraction containing about 70 % of the ejaculate volume (Tavener and Dunkin, 1996). Epididymal semen volume is difficult to determine due to blood contamination during the process of harvesting the semen.

#### ***2.4.3.2 Semen pH***

The semen pH indicates the acidity or alkalinity of semen sample. Normally, the pH of boar semen is alkaline because of the seminal vesicles (accessory gland). An alkaline pH protects the spermatozoa from the acidity of the vaginal fluid, while an acidic pH indicates problems regarding seminal vesicle function (Essig, 2007). The pH of raw boar semen varies between 7.0 and 7.5. Major changes in boar semen pH can result in sperm damage, infertility, or sperm mortality (Purdy, 2006).

#### **2.4.3.3 Semen concentration**

Semen concentration is expressed as the number of spermatozoa cells per ml and must be known for each ejaculate to be used to maximize the number of artificial insemination (AI) units containing a given number of motile spermatozoa units (AI dose). Assessing spermatozoa concentration can be done in both raw and diluted semen samples and requires <10 µl raw ejaculate in the disposable micro cuvette. The self-loading micro-cuvette also ensures accurate sample volume. It has a LED light source for stable calibration and the digital display shows the semen concentration in million sperm/ml and the final reading is based on the average of multiple readings. The machine automatically resets to zero after each sample. It is 110 V or battery operated, making it portable (Rigby *et al.*, 2001). The total spermatozoa concentration lies between 10 and 100 billion spermatozoa cells per ejaculate (Frunza *et al.*, 2008). Hafez (1993) reported the mean spermatozoa concentration of boars ranges from 0.1 to 0.2 x 10<sup>9</sup> spermatozoa/ml.

#### **2.4.3.4 Spermatozoa motility**

Motility is an important characteristic in predicting the fertilizing potential. Changes in spermatozoa movement patterns can reflect physiological events within the sperm (Masenya, 2012). A percentage of live spermatozoa can be estimated per their motility (Bjoerndahl *et al.*, 2004). Kozdrowki *et al.* (2007) reported that regard the motility of spermatozoa as one of the most important indicators of the semen quality assessment. Hafez and Hafez. (2000) reported that spermatozoa motility involves a subjective estimation of the viability of the spermatozoa and their motility. Spermatozoa motility is commonly believed to be one of the most important characteristics used when evaluating the fertility potential of ejaculated spermatozoa (Hashida and Abdullah, 2003).

Moc and Graham, (2008) referred to this visual estimation of the percentage of motile spermatozoa in a semen sample as the most general laboratory semen assay performed. The simplest way to evaluate sperm motility is by estimating the number of motile sperm under a light microscope or using phase contrast microscopy (Shiple, 1999). Objective Computer Aided Sperm Analysis® (CASA®) systems have become commercially available, but these systems are not frequently used in commercial AI centres because of the high investments costs (Verstegen *et al.*, 2002). Total spermatozoa motility is generally defined as the ratio of motile cells to the total cell population, expressed as a percentage. Progressive sperm motility is the number of spermatozoa cell moving in a forward and in a straight – line direction. Straight-line/ progressive velocity (VSL) is the velocity on a straight-line distance between the beginning and the end of the track. Semen ejaculates with at least 70 % gross motility should be used for further processing. This is important because spermatozoa motility and viability normally decrease during storage. If ejaculates are used shortly after collection, samples exhibiting at least 60 % motility can be used for AI.

#### ***2.4.3.5 Spermatozoa morphology***

The structure, or morphology, of spermatozoa has been studied extensively using light and electron microscopy techniques. The spermatozoa are a highly structured cell that is streamlined to deliver DNA to the oocyte. Spermatozoa morphology seems to be one of the most important qualitative characteristics of semen and can serve as an indicator of some disorders in the process of spermatogenesis (Kuster, 2005). Primary morphological abnormalities, those that affect sperm head region, because of a disturbance in spermatogenesis, contribute a significant role in determining the population of sperm that reach the site of fertilization. Reminders of cytoplasm,

proximal or distal droplets and small tail abnormalities are defined as secondary abnormalities and can be compensated for by semen dose (Donadeu, 2004). There are numerous factors that affect fertility of boars. These include selection, age of boar, ambient temperature, photoperiod, rhythm of semen collection, social contact with other pigs, accuracy of semen processing and nutrition.

## **2.5 Factors affecting boar fertility**

Various factors influence fertility of boars. These include selection, age of a boar, ambient temperature, photoperiod, rhythm of semen collection, social contact with other pigs, accuracy of semen processing and nutrition.

### **2.5.1 Selection**

Improvements in reproductive characteristics can be achieved by selection of animals with superior genetic make-up as the parents for the next generations. Selection of productive sow contributes most to the revenue and cost of pig enterprise. To improve profits obtained from a pig enterprise, the number of piglets born alive should be increased. The survival for piglets until weaning is important (Dube *et al.*, 2012). Productivity per farrowing is usually considered the basis for evaluating the genetic merit of animals in a herd. Genetic selection for sow productivity, genetic parameters should be estimated. Estimated genetic parameters for sow productivity traits are generally of low heritability (Roehe *et al.*, 2009). The heritability estimates give an indication of the rate of genetic progress that can be achieved when genetic selection.

Very few observations recorded on the Windsnyer pig, which is bred according to traditional standards are number of these pigs are decreasing genetic selection is limited. The productive traits

like litter size and mortality rates important for measuring efficiency of selection practices (Pathiraja, 1987). When selecting a boar for a breeding program, factors such as origination from a specific disease-free herd, performance, soundness and conformation, age of puberty and other pertinent parameters related to reproduction should be considered. Selection process, boars from large litters (>10 piglets) that reach puberty early 5 months tend to produce highly productive piglets.

### ***2.5.2 Age of a boar***

A boar fertility is diminished by advanced age, especially when they are over three years of age. Physiological maturation in boars after birth is an ongoing process. Between 1-2 months of age, mounting behaviour is first observed in young boars. At three months of age, there is a second period of germ cell division and rapid increase in the testes to body weight ratio. At four months of age, sperm first appear in the seminiferous tubules and erection can be accomplished in the ejaculate. Over the next 6-18 months, the testes increase in size and both semen concentration and ejaculate volumes continue to increase. By 18 months of age, no appreciable improvements in fertility are observed and the boar is considered fully mature (Levis *et al.*, 1997). Most boars reach puberty (ability and willingness to ejaculates fertile spermatozoa) between 5 and 8 months of age. Spermatozoa may be found in the testes much earlier (110 -125 days of age) than this, but there is some delay before the sperm are able to fertilize ovary and the boar develops the coordinated pattern of sexual behaviour necessary for successful copulation (Levis *et al.*, 1997).

#### ***2.5.4 Ambient temperature***

The major contribution to the semen variation is via environment (Curtis, 1983; Cupps, 1991; Bearden and Fuquay, 1997). Sows and boars both suffer from acute and persistent exposure to elevated ambient temperature. Infertility can be relatively short term or permanent disability from which the animal will never recover. Pigs feel heat based on temperature and humidity. The effect of heat stress on reproduction has been related to ambient temperatures more than 27<sup>0</sup> C. There is also evidence that boars exposed to ambient temperatures more than of 29<sup>0</sup>C have lower spermatozoa output and poorer spermatozoa motility and increased morphological abnormalities (Parrish *et al.*, 2016).

Pigs have low capacity for increased sweating when the ambient temperature increases e.g. from 23 to 34<sup>0</sup>C, which contributes to the close relationship between environmental temperature and scrotal and testicular temperatures during such periods (Stone, 1981). Lowered fertility and lowered spermatozoa concentration and decreased ejaculate volume have been found in boars during or shortly after the warm summer period in several countries in Europe and North America (Thibault *et al.*, 1966). In tropical areas, such as Thailand temperature has a significant negative effect on both the ejaculate volume, spermatozoa concentration and the morphology of the spermatozoa (Suriyasomboon *et al.*, 2004). In most studies, an increased proportion of abnormal spermatozoa has been reported after heat treatment but the results vary among boars and are also related to the different regimes for causing heat stress (Larsson and Einarsson, 1984). An acute rise in rectal temperature related to the detrimental effects on the testes was observed in some heat exposed boars (Cameron and Blackshow, 1980). This indicates that the stress, imposed by elevated temperature, may not be of the same magnitude for all boars.

### **2.5.5 Photoperiod**

Photoperiod is important in the regulation of boar reproduction. The role of photoperiod on semen quality is, however, still controversial. Berger *et al.* (1980) reported that boars kept under natural light plus artificial light supplementation (10-500 lux) to maintain constantly 15h of light/day from 11 weeks of age until puberty (24-26 weeks). Faster sexual maturation and a higher libido than boars receiving only natural light during that period (15h at 11 weeks to 9h at the end of the trial). However, there was no effect on semen quality. Sancho *et al.* (2006) showed that submission of boars to either 24h of artificial light or 24h of complete darkness for three months reduce semen volume and concentration. In the latter study, there was a reduction in semen volume and concentration after one month of exposure to 24h of light or of complete darkness but, after three months, semen volume and concentration seemed to return to the values before treatment. Other studies, photoperiod did not affect spermatozoa motility or vitality (Trudaeau and Sanford, 1986). There is no current literature available on effect of photoperiod on boar fertility.

### **2.5.6 Rhythm of semen collection**

Semen collection from boars in AI-centres is performed approximately twice per week (Vyt *et al.*, 2007). A high frequency of collection has a negative effect on semen quality because spermatozoa is forced to rapidly pass from caput to cauda of the epididymis thus having insufficient time for epididymal maturation (Strzezek *et al.*, 1995). It was also demonstrated that submitting boars to collection four days in a row affected the re-absorption/ secretion pattern of fluids in the lumen of epididymis (Pruneda *et al.*, 2005). This imbalance in the secretion of fluids resulted in an increase of abnormal spermatozoa and a reduction in spermatozoa motility.

### ***2.5.7 Social contact with other pigs***

Although adult boar are housed in individual pens, group housing of growing boars is beneficial for subsequent reproductive performance. Groups of eight boars from 30 kg housed in pens of 4 m x 4.3 m until they successfully completed two mountings, had on average stronger legs for jumping, higher libido, earlier accomplishment of the first mating and high sperm counts compared to boars housed individually (Hacker *et al.*, 1994). Mature boars should not be kept together as they will fight and cause each other severe injuries. Current European Union guidelines from the Scientific Veterinary Committee suggests that keeping boars in a separate pen but in visual contact with females is an appropriate solution. It is much easier to heat-check sows by fence-line boar contact instead of placing the boar in the sow pen for full boar contact (Umesiobi, 2010). Fence-line contact with a boar is inadequate to stimulate puberty in most sows. Full boar contact is needed when sows are taken to a high stimulation area that only houses boars (Umesiobi, 2007).

### ***2.5.8 Accuracy of semen processing***

Semen collection is normally performed by the gloved handed technique (Knox *et al.*, 2008). Polyvinyl gloves can be used, latex gloves should be avoided as these are toxic for the spermatozoa. The end of the penis is grabbed firmly with gloved hand and the collection process is initiated with firm pressure to the spiral end of the penis with the hand so that the penis cannot rotate. This process imitates the pressure applied by the corkscrew shape of the sow's cervix. A pre-warmed (38<sup>0</sup> C) collection container is used to avoid rapid cooling of the ejaculate (Maes *et al.*, 2011). The top of the container is covered with cheesecloth to filter out gel portion of the semen. The first part of the ejaculate (pre-spermatozoa) should be discarded. It is a clear, watery fluid and does not contain spermatozoa but it may have a high bacterial count. The spermatozoa-

rich fraction should have collected (40 to 100 ml). It is very chalky in appearance and contains 80-90% of all spermatozoa cells in the ejaculate. Once the spermatozoa-rich fraction is complete, the remainder of the ejaculate is again a clearer, watery fluid which should not be collected (70 to 300 ml). The ejaculation lasts up to 5 to 8 min, but may continue up to 15 min. About 100 to 300 ml of semen is routinely collected.

After collection, the filter with gel should be discarded and the collection container should be placed in warm water. Spermatozoa motility and vitality will be only be retained for few hours (Johnson *et al.*, 2000). To prolong spermatozoa survival their metabolic activity must be inhibited by chemical inhibitors or by lowering the temperature and therefore the ejaculate needs to be extended shortly after collection (Johnson *et al.*, 2000). Compared to semen of other animal species, boar spermatozoa are very susceptible to temperatures below 15<sup>0</sup> C, due to a different composition of the phospholipids in their membrane (De Leeue *et al.*, 1990). The temperature of the ejaculate now of collection is approximately 37<sup>0</sup> C and of approximately 32-35<sup>0</sup> C at arrival in the laboratory where it is processed (Waberski, 2009). Fast cooling of the ejaculate from body temperature to temperature below 15<sup>0</sup> C will result in lipid phase separation that will alter the spermatozoa membrane permeability with subsequent loss of spermatozoa vitality (Johnson *et al.*, 2000). These changes in membrane permeability will result in calcium influx into the spermatozoa that would stimulate capacitation like changes (Petrunkina *et al.*, 2005).

### **2.5.9 Nutrition**

Semen quality and quantity are major factors of concern in reproduction and can be adversely affected by malnutrition (Brown, 1984). Cupps, (1991) summarised that deficient nutrient intake

for example iodine, zinc, cobalt, Vitamin A, Vitamin E and minerals are associated with reduction in semen quality in terms of sperm morphology, concentration and motility.

Nutrition affects boar libido, spermatozoa output and semen quality. Therefore, reproductive performance can be significantly decreased if a proper nutritional programme is not implemented. Breeding performance is reduced due to decreased blood levels of estradiol-17 $\beta$  (Louis *et al.*, 1994). Short term restrictions in feeding level or nutrient intake have, however, minimal effects on libido (Dutt and Barnhart, 1959; Ju *et al.*, 1985; Kemp *et al.*, 1989). Potentially more significant is the detrimental effect of over-conditioning boars on subsequent libido. Excessive body weight gain reduces the activity level in pigs by making them fatter and more lethargic, and may decrease the physical ability of a boar to mount a sow by reducing locomotive soundness and balance (Westendorf and Richter, 1977).

The process of producing spermatozoa in the testis and allowing them to mature in the epididymis takes approximately six weeks. Therefore, nutritional effects cannot be observed when examining spermatozoa characteristics for at least 42 days after implementing a nutritional change. Providing a lower plane of nutrition (between 50 – 70 % of requirements) has resulted in reduced semen volume and total sperm production (Kemp *et al.*, 1989; Beeson *et al.*, 1953), while increasing the nutrient levels back to required levels returned semen volume and sperm production to normal.

#### ***2.5.9.1 Effect of energy levels in the diet***

Overfeeding and excess body weight contributes to the increased incidence of feet and leg problems as well as the reduction of libido in boars. Penny and Guise, (1989) argued that excess

body weight is the major reason for culling boars. Maximum semen volume, spermatozoa concentration and doses of semen were obtained from boars aged between 24 and 29 months, while boars under nine months of age demonstrated lower production (Kennedy and Wilkins, 1984).

Energy requirements for the boars can be broken into several categories: maintenance, weight gain, mating activity and sperm production. The maintenance requirement was calculated as 415 kJ ME/kg live weight<sup>0.75</sup>/d for young boars, old boars (763 kJ ME/ kg live weight<sup>0.665</sup>) (Close and Roberts, 1993). Principles of nutrient partitioning and few assumptions were used to create equations that would estimate energy requirements for growth, mating activity and spermatozoa production (Kemp, 1991) and calculate a total energy requirement for boars. Since the energy requirements for mating activity and spermatozoa production are small, they are often ignored when estimating energy needs of the working boar. A prolonged period of restriction of energy, protein and vitamin E adversely affects libido and semen quality in boars. The nutrient requirement of the boar and dietary specification are known, and separate boar diets is essential. The addition of vitamin E which has resulted in improved semen quality and fertilisation rate (Monsalve *et al.*, 2004).

#### **2.5.9.2 Effect of vitamin E**

Vitamin E ( $\alpha$ -tocopherol) is a lipid soluble, chain-breaking antioxidant. Specifically,  $\alpha$ -tocopherol inhibits peroxidation of polyunsaturated fatty acids (PUFA), which is especially important in sperm due to their high PUFA content (Bolle *et al.*, 2002). Vitamin E have effect on spermatozoa maturation and quality. The antioxidants are thought to assists reduce stress and membrane damage. There is little evidence for improved reproductive performance related to the antioxidant level being fed to the boar. However, in studies where elevated levels were fed, viability of the

spermatozoa was enhanced. Vitamin E requirements vary with age of pigs. The NRC, (1998) has estimated 44IU per kg of diet is required for boars. The Agricultural Research Council (ARC, 1981) has suggested that growing pigs require Vitamin E levels of between 20 to 50 IU per kg of diet. In boars, it was reported that vitamin E enhanced fertility when supplementation was raised from 40 to 80 IU per kg feed (Westendorf and Richter, 1977). Vitamin E requirements are exceedingly difficult to determine because of the interrelationships with other dietary factors. It is generally accepted that Vitamin E supplementation not only improve production semen volume, but also spermatozoa concentration (McDowell, 1996). The amount of vitamin E needed to maintain adequate growth and reproduction would not necessarily be enough to ensure optimal immune function.

## **2.6 Reactive oxygen species and male infertility**

Reactive oxygen species (ROS) are free radicals that are highly active oxidants and include superoxide anion, hydrogen peroxide, peroxy and hydroxyl radicals, ROS can be produced by immature spermatozoa and leukocytes (Whittington and Ford, 1999). Agarwal *et al.* (2003) reported that low levels of ROS are beneficial in stimulating sperm capacitation, enhance zona pellucida binding and promote acrosome reaction. Then high levels of ROS are harmful and lead to lipid peroxidation of spermatozoa plasma membrane and DNA fragmentation. The increased lipid peroxidation is associated with impaired spermatozoa motility and diminished capacity for spermatozoa-oocyte fusion. One of the consequences of uncontrolled oxidative stress in the cells, tissues and organs injury caused by oxidative damage. High levels of free radicals or ROS can inflict direct damage to lipids. The primary source of endogenous ROS production are the mitochondria, plasma membrane, endoplasmic reticulum and peroxisomes (Moldovan and

Moldovan, 2004) through a variety of mechanisms including enzymatic reactions. The levels of ROS are normally limited by various antioxidant defence mechanisms, such as  $\alpha$ -tocopherol that is present within the seminal plasma and plasma membrane (Agarwal *et al.*, 2003). There is a need of supplementing antioxidant to scavenge the free radicals.

### **2.6.2 Lipid peroxidation in spermatozoa**

Lipid peroxidation can be described generally as a process under which antioxidants such as free radicals or non-radicals species attack lipids containing carbon- carbon double bond(s), especially polyunsaturated fatty acids (PUFAS) that are involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxy radicals and hydroperoxides. Naher *et al.* (2013) reported that lipid peroxidation in boar semen is one of the most important factors causing infertility in pigs as well as causing decreased spermatozoa quality during the storage of semen from boars. Therefore, the mechanisms by which ROS disrupt spermatozoa function probably involve the peroxidation of PUFA in the spermatozoa plasma membrane. More importantly, biological free radicals have been implicated to cause major causes of poor performance and low productivity in a high intensive production system (Nwangu, 2012).

### **2.7 Summary**

Numerous factors influence semen and fertility on boars. Pig development and reproductively is affected by several factors such as environmental, nutritional management and genetic factors. These factors have positive impact on the animal overall productivity. Supplementing antioxidants in pig diets can provide natural boost towards free radical control and the balance between the antioxidants in the diet, development, gastro-intestinal tract, semen and tissues is an important

determinant of health status. This can provide the natural systems to improve animal health, welfare and productivity.

Boar spermatozoa are sensitive to peroxidative damage due to high content of unsaturated fatty acids. Little, if any, is known about the effect of  $\alpha$ -tocopherol supplementation on spermatozoa quality of Windsnyer boars. Therefore, the aim of the current study was to determine the response in semen fertility to increasing  $\alpha$ -tocopherol supplementation of Windsnyer boars. The possible influence of dietary supplementation of Windsnyer boars with  $\alpha$ -tocopherol inclusion was also investigated.

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### CHAPTER 3: Inclusion levels of $\alpha$ -tocopherol supplementation on growth performance and testicular development of Windsnyer boars

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#### Abstract

The study was conducted to determine the response in growth performance and testicular development of Windsnyer boars to  $\alpha$ -tocopherol supplementation. Twenty Windsnyer boars aged 3 months old with an average body weight  $\pm$  (standard deviation) of  $19.5 \pm 2.67$  kg were selected. Each pig was housed individually in a 1.54 x 0.8 m pen in environmentally controlled house with the temperature ranging from 22 to 25<sup>0</sup>C. Pigs were kept until they reached an average body weight of 57 kg. Five boars were randomly assigned to each diet containing inclusion levels of  $\alpha$ -tocopherol 0, 40, 70 and 90IU. Polynomial regression analysis was used to determine the relationships between  $\alpha$ -tocopherol inclusion level and growth performance and testicular development. Supplementation of  $\alpha$ -tocopherol inclusion levels had a quadratic relationship with body weight of Windsnyer boars. There was a quadratic decrease ( $P < 0.05$ ) in average daily gain (ADG) as  $\alpha$ -tocopherol levels increased. The equation was  $y = -0.0083x^2 + 0.7786x - 0.08$ . There was no relationship between  $\alpha$ -tocopherol and testicular development and testicular tissues ( $P > 0.05$ ). There was a tendency ( $P = 0.09$ ) of quadratic increase in seminiferous tubule with increase in  $\alpha$ -tocopherol inclusion level. The testicular tissues were at the normal range for boars. In conclusion,  $\alpha$ -tocopherol inclusion levels has a quadratic relationship with average daily gain and body weight of Windsnyer boars. Testis and testicular tissues remain at normal range were not influence by  $\alpha$ -tocopherol inclusion.

**Keywords:** feed intake, feed conversion ratio, growth rate

### 3.1 Introduction

Windsnyer pigs are predominantly found in South Africa, Mozambique and Zimbabwe (Halimani *et al.*, 2010). Windsnyer pigs are shunned because of their slow growth rate, gaining about 0.2 kg/day (Kanengoni *et al.*, 2004). The breed is discriminated because compact carcasses, making it difficult to cut into specialised meat portions (Chimonyo *et al.*, 2010). Utilisation of Windsnyer pigs assists in conservation of such valuable superior genetic resources (Chimonyo *et al.*, 2010). Nonetheless, Windsnyer pigs play crucial role to rural livelihood. They have desirable traits for purposes that suit small-scale production system such as hardiness and adaptability to harsh climatic conditions. Windsnyer pigs are generally early maturing and reach puberty at three months of age. They are able to utilise fibrous diets more efficiently and strive to survive under poor plane of nutrition. Chimonyo *et al.* (2007) highlighted that Windsnyer pigs have a low energy requirements for maintenance. There is, therefore, a need to conserve Windsnyer pigs. To do this information on testicular development particularly to boars is important.

Vitamin E ( $\alpha$ -tocopherol) is a lipid soluble and essential nutrient that is involved in number of biochemical and physiological processes. Deficiency of vitamin E compromises growth and reproductive efficiency. Alpha-tocopherol is a chain-breaking antioxidant together with ubiquinol, carotenoids, vitamin A, ascorbic acid, uric acid and glutathione peroxidase (GPx). Alpha-tocopherol is the most effective natural free radical scavenger. Alpha-tocopherol is a potent antioxidant and was demonstrated to decrease lipid oxidation (Monahan *et al.*, 1992). It inhibits peroxidation by scavenging peroxy radical intermediates and keeping the chain length of the propagation reaction as small as possible. Hydroperoxides, which are produced in the reaction of

$\alpha$ -tocopherol and the peroxy radical, are toxic and if not removed, can impair the structure and function of membranes

Slow growth rate of Windsnyer pigs is their major weakness. It is not clear whether  $\alpha$ -tocopherol inclusion improves growth rate of boars. McDowell *et al.* (1996) suggested that vitamin E is essential for growth and integrity of tissues. The authors indicated that there is a relationship between the growth of testicular components, absolute testis weight and body weight in boars. Although growth of animals is largely accounted for by energy and protein consumption, it is possible that  $\alpha$ -tocopherol supplementation may further enhance average daily gain and feed efficiency in pigs (Asghar *et al.*, 1991). Furthermore, spermatogenic tissues increase with age, body weight and testis weight in boars (Harder *et al.*, 1995; Ugwu *et al.*, 2009). Research in rats showed that deficiency of vitamin E resulted in the degeneration of the testes, resulting in permanent sterility (Bearden and Fuquay, 1997). The objective of the current study was, therefore, to determine the relationship of growth performance and testicular development of Windsnyer boars to  $\alpha$ -tocopherol supplementation. It was hypothesized that  $\alpha$ -tocopherol inclusion influences growth performance and testicular development of Windsnyer boars.

## **3.2 Materials and methods**

### **3.2.1 Study site**

The study was conducted at the Pig Research Unit of Agricultural Research Council (Gerplasm Conservation and Reproductive Biotechnologies Unit), Irene (Pretoria), South Africa. The Agricultural Research Council-Irene campus is located at 25°55' S and 28°12' E in Pretoria, South Africa and situated on the highveld with altitude of 1525m above sea level. Approximate mean

annual rainfall of 715mm is received with mean annual temperature of 17.3<sup>0</sup>C and mean annual humidity of 75 %.

### **3.2.2 Pigs, diets and experimental design**

Twenty Windsnyer boars aged three months with average body weight ( $\pm$  standard deviation) of  $19.5 \pm 2.67$  kg were randomly selected from ARC-API pig breeding section. Pigs were housed individually in 1.54 x 0.8 m pens in an environmentally controlled house with temperature ranging from 22 to 25<sup>0</sup>C. Four diets were formulated to be (15.1 MJ AME/kg) iso-energetic and (155 CP/kg) iso-nitrogenous containing different amounts of  $\alpha$ -tocopherol 0, 40, 70 and 90 IU d- $\alpha$ -tocopherol/kg<sup>-1</sup> DM. Five pigs were randomly allocated to one of the four dietary treatments. Pigs were fed *ad libitum* and water was made available through nipple drinkers.

The boars were fed daily at 0730h. The spilt feed was collected back into the feeding trough while the wet feed was air dried and added to the left-over feed at the end of the week to determine weekly feed intake. All boars were dipped using the Triatix<sup>®</sup> (2 % Pig Pour on) prior to the start of the experiment. On the day of start the feeding trial, pigs were weighed individually with an electronic weight indicator KM3 (Rudoweight, Barlo) to obtain initial body weights. Growth performance trial was conducted for duration of 3 months. Diets were formulated to meet boar requirements by using Format international- feed formulation software solution (FI-FFSS). The formulated diets are given in Table 3.1.

**Table 3.1: Ingredient composition and chemical composition of the experimental diets fed****Windsnyer boars**

<b>Ingredients</b>	<b>Content (g/kg DM)</b>
Hominy chop	197
Wheat bran	71
Soyabean meal cake	161
Maize	538
Monocalcium phosphate	7
Feed lime	17
Lysine	2
Salt	2
Premix	2
<b>Chemical analyses</b>	
Crude protein (g/kg DM)	155
Crude fibre (g/kg DM)	61
Digestible energy (MJ/kg DM)	135
Ether extract (g/kg DM)	38.6
Calcium (g/kg DM)	9.1
Phosphorus (g/kg DM)	5.2

### **3.2.3 Measurements**

#### **3.2.3.1 Average daily feed intake**

The determination of weekly feed intake (WFI) was done every Friday throughout the experiment. The WFI was obtained by weighing back the leftover feed in the bucket, rough as well as any amount of feed spilt during the week for each pig and the sum was subtracted from the total weight of feed allocated to each pig. All this was done using table top weighing scale (Changzhou, Yubo Electronic). The summation of WFI for each pig was considered as total feed intake for each pig. The WFI was divided by 7 to estimate the average daily feed intake for each pig.

The determination of weekly feed intake (WFI) was done every seventh day throughout the experiment. The WFI was obtained by weighing back the leftover feed in the bucket, rough as well as any amount of feed spilt during the week for each pig and the sum was subtracted from the total weight of feed allocated to each pig. All this was done using table top weighing scale (Changzhou, Yubo Electronic). The summation of WFI for each pig was considered as total feed intake for each pig. The WFI was divided by 7 to estimate the average daily feed intake for each pig.

#### **3.2.3.2 Average daily gain**

During the study, pigs were weighed individually on a weekly basis to determine daily body weight gain using electronic weight indicator scale. The mean daily weight gain was calculated by final body weight minus initial body weight dividing by number of days. Body weights (BW) were recorded on weekly basis prior to feeding to estimate average daily gain (ADG). The boars were weighed from three months of age (start of the experiment). The experiment ran for 18 months.

### **3.2.3.3 Feed conversion ratio**

Feed conversion ratio, defined as the quantity of feed (kg) consumed to gain a unit weight was calculated as a ratio of total feed consumed to total weight gain for each pig. Feed conversion ratio (FCR) was estimated by dividing the ADG by the ADFI.

### **3.2.3.4 Testicular development**

Pigs weighing  $57.2 \pm 1.67$  kg (mean  $\pm$  standard deviation) were transported simultaneously at 0730h at the ARC–Irene abattoir, where they were provided with fresh drinking water, kept calm and then slaughtered. At the abattoir, standard pre-slaughtering procedures were followed. Each pig was electrically stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for six seconds. Electrical stunner electrodes were positioned at the base of each ear. Exsanguination followed within 10 seconds after stunning. Dehairing and evisceration were done based on abattoir's standard operating procedures. The testicle and epididymis from each pig was removed. The length, width of the left and right testis, left and right epididymis were measured using venier calliper. The weight of testicles was taken using a sensitive weighing scale. The right and left epididymis was trimmed off the body of the testis.

### **3.2.3.5 Histological evaluation**

The testes samples were weighed and fixed in 10 % formaldehyde® before evaluation of seminiferous tubules, Sertoli cells, spermatogenic, Leyding cells and germinal epithelium. Sections of 4 mm were cut with a Leica sliding microtome and the slides were stained with haematoxylin-eosin. The average tubular diameter determination per sample was performed by randomly measuring 15 cross sections. The count was performed using a BX51 Olympus optical

microscope and a digital haemocytometric cell counter. For the quantitative distribution of spermatogenic cell types, all sections were evaluated by the same person. A photo-micrographic software, Phoenix Micro Image Analysis (2003) version 1.33, was used to project the slide on the compute for clear assessment.

### **3.2.6 Statistical analyses**

A polynomial regression (PROC REG) procedure of (SAS, 2008) was used to determine the relationships between  $\alpha$ -tocopherol inclusion and growth performance and testicular development.

The model that was used was:

$$Y = B_0 + B_1A + B_1A^2 + E$$

Y = is the response variable (body weight, average daily feed intake, average daily gain, feed conversion ratio, testicular development and histology)

$B_0$  = is the intercept

$B_1A$  = linear regression component

$B_1A^2$  = quadratic regression component

E = is the error

### **3.3 Results**

#### **3.3.1 Growth performance**

The relationship between growth performance of Windsnyer boars and  $\alpha$ -tocopherol inclusion level is indicated in Table 3.2. There was no relationship between the  $\alpha$ -tocopherol inclusion levels and ADFI and FCR ( $P > 0.05$ ).

**Table 3.2: Relationship between growth performance characteristics of Windsnyer boars fed diets containing inclusion levels of  $\alpha$ -tocopherol**

<b>Trait (kg)</b>	<b><math>\alpha</math>-tocopherol inclusion (IU)</b>				<b>SEM</b>	<b>P value</b>	
	<b>0</b>	<b>40</b>	<b>70</b>	<b>90</b>		<b>Linear</b>	<b>Quadratic</b>
ADFI (kg)	1.41	1.42	1.41	1.42	0.00	0.897	0.195
ADG (kg)	0.15	0.16	0.14	0.16	0.02	0.890	0.023
FCR	13.91	9.85	12.61	10.68	1.29	0.248	0.825

Significantly different at ( $P < 0.05$ ). Abbreviations: SEM = standard error mean; ADFI= average daily feed intake; ADG= average daily gain; FCR= feed conversion ratio.

The quadratic relationship between body weight of Windsnyer boars and  $\alpha$ -tocopherol level as shown in Figure 3.1. There was a quadratic body weight response on  $\alpha$ -tocopherol level. As the  $\alpha$ -tocopherol level increase the body weight increase. The relationship between ADG and  $\alpha$ -tocopherol inclusion is shown in Figure 3.2. There was a quadratic influence of  $\alpha$ -tocopherol on ADG. The ADG increase at low level inclusion  $\alpha$ -tocopherol and started to decrease at high  $\alpha$ -tocopherol level. The relationship equation between ADG and  $\alpha$ -tocopherol level was ( $y = -0.0083x^2 + 0.7786x - 0.08$ ;  $P < 0.05$ ).

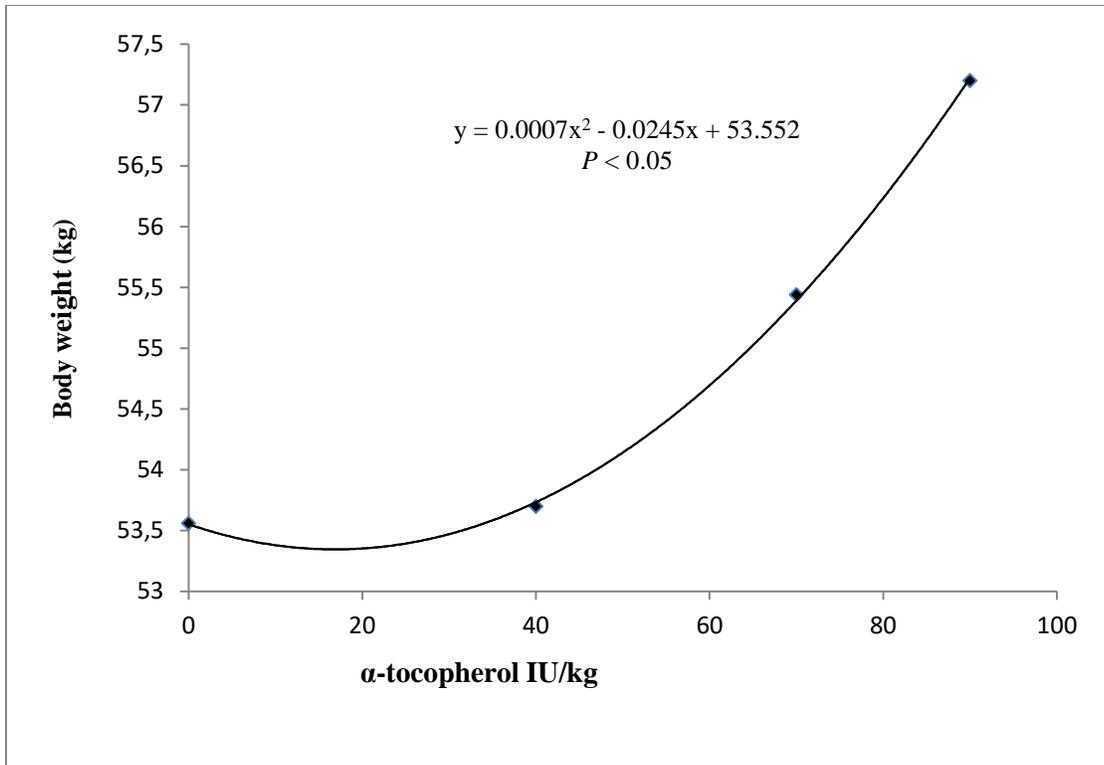
### **3.3.2 Testicular development**

The response of dietary  $\alpha$ -tocopherol inclusion on testicular characteristics of Windsnyer boars is given in Table 3.3. Supplementation of  $\alpha$ -tocopherol had no relationship to testicular development of Windsnyer boars ( $P > 0.05$ ). There was no relationship between  $\alpha$ -tocopherol inclusion and the development of both left and right testicles ( $P > 0.05$ ). Also, no relationship observed between left and right epididymis of  $\alpha$ -tocopherol inclusion. There was no relationship between the weight of testis and epididymis and  $\alpha$ -tocopherol inclusion in Windsnyer boars ( $P > 0.05$ ).

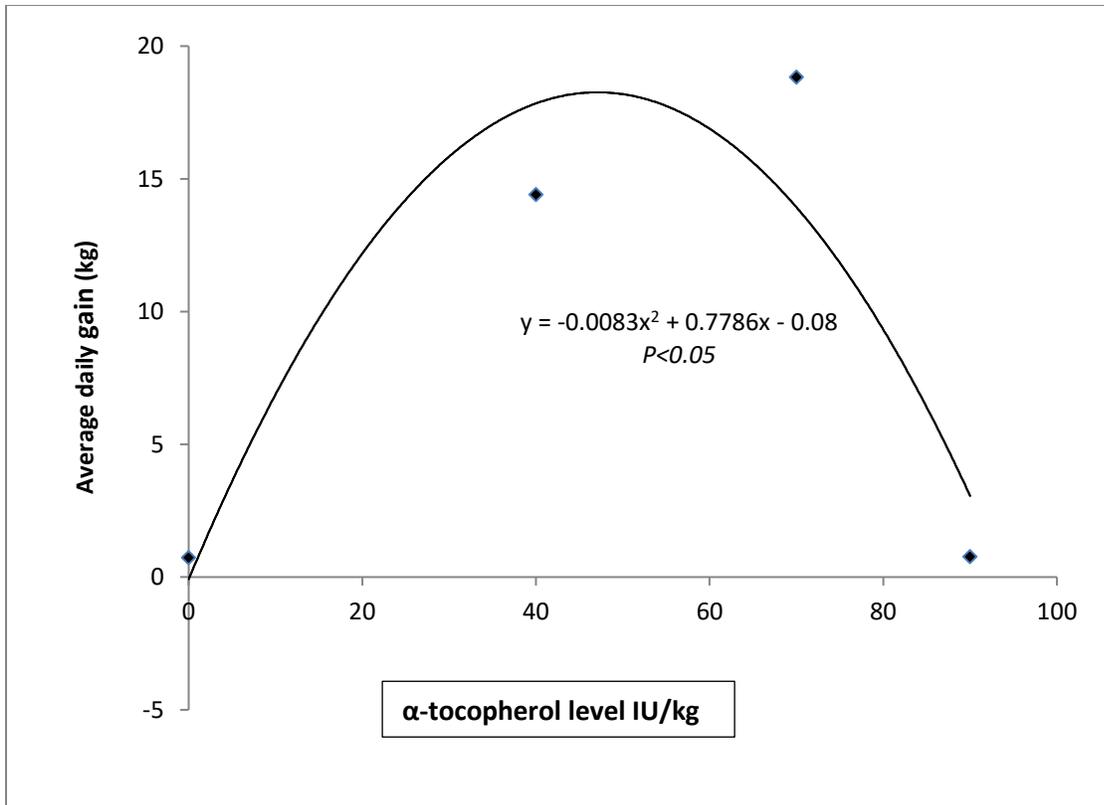
### **3.3.3 Histological indexes of testicles**

The influence of  $\alpha$ -tocopherol inclusion levels on histological indexes of testicle of Windsnyer boars is shown in Table 3.4. There was no influence of  $\alpha$ -tocopherol on histological indexes of testicles ( $P > 0.05$ ). There was, however, a tendency of quadratic increase of seminiferous tubule response to supplementation of  $\alpha$ -tocopherol inclusion level ( $P = 0.09$ ). Response of  $\alpha$ -tocopherol on histological structure of testis is shown in Figure 3.3. The density of Leyding cells was not affected by inclusion levels of  $\alpha$ -tocopherol inclusion ( $P > 0.05$ ). However, the density of Leyding

cells tended to be numerically higher in pigs supplemented with  $\alpha$ -tocopherol inclusion. There was no relationship between histological indexes and  $\alpha$ -tocopherol inclusion ( $P > 0.05$ ).



**Figure 3.1: Relationship between  $\alpha$ -tocopherol and final body weight of Windsnyer boars**



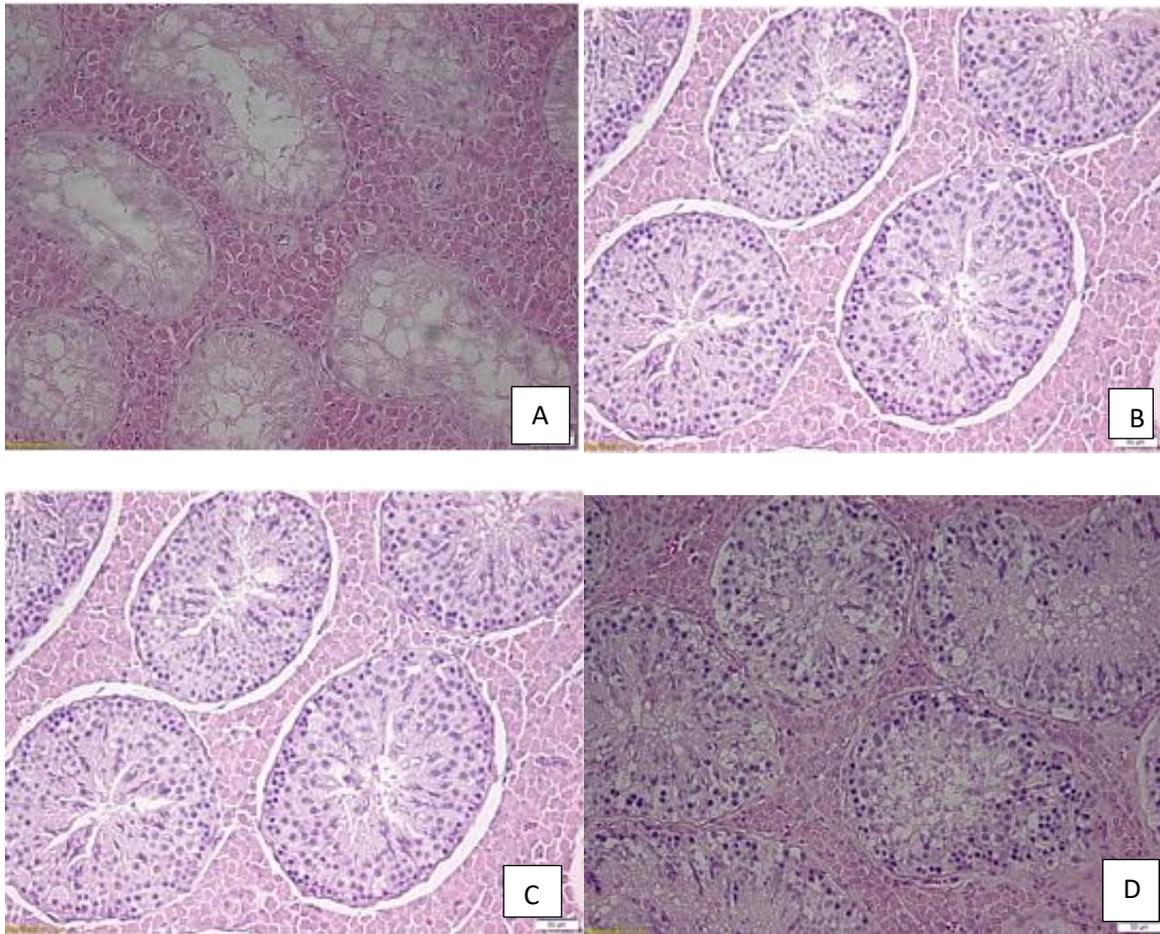
**Figure 3.2: Relationship between average daily gain and  $\alpha$ -tocopherol inclusion**

**Table 3.3: Influence of dietary  $\alpha$ -tocopherol inclusion on testicular development for Windsnyer boars**

Measurement	Levels of $\alpha$ -tocopherol (IU)				SEM	P-value	
	0	40	70	90		Linear	Quadratic
Left length (mm)	83.2	93.0	91.4	91.0	4.46	0.182	0.748
Left width (mm)	49.0	50.3	52.2	52.6	2.51	0.909	0.927
Right length (mm)	83.0	93.0	84.6	82.5	4.71	0.220	0.443
Right width (mm)	49.4	53.6	51.8	51.7	2.93	0.541	0.557
Right epididymis (mm)	150.0	150.0	147.0	149.7	1.83	0.541	0.557
Left epididymis (mm)	147.0	150.0	148.7	147.2	2.13	0.885	0.187
Left testicular weight (g)	92.6	128.3	111.3	111.9	16.40	0.413	0.914
Right testicular weight (g)	93.5	125.5	113.7	123.2	14.00	0.139	0.487
Left epididymis weight (g)	26.5	36.4	28.9	26.3	3.53	0.914	0.991
Right epididymis weight (g)	26.8	38.7	32.1	29.4	3.09	0.591	0.267

**Table 3.4: Influence of  $\alpha$ -tocopherol inclusion on histological indexes of testicles.**

Tissues	$\alpha$ -tocopherol levels				SEM	P value	
	0	40	70	90		Linear	Quadratic
Seminiferous tube area (mm <sup>2</sup> )	0.06	0.08	0.06	0.07	0.01	0.736	0.097
Density of Sertoli cells <sup>1</sup>	6.53	12.93	4.86	6.00	2.65	0.697	0.756
Density of spermatogenic cells <sup>2</sup>	58.46	62.06	54.13	59.86	7.02	0.889	0.963
Density of Leyding cell number per 0.02 mm	37.93	75.66	49.33	52.53	7.64	0.556	0.218
Thickness of germinal epithelium ( $\mu$ m)	87.00	102.00	98.66	97.16	8.50	0.369	0.177



**Figure 3.3: Response of  $\alpha$ -tocopherol on diet of Windsnyer boars on histological structure of testis (100x magnification); A- 0 IU; B-40 IU; C-70 IU and D-90 IU.**

### 3.5 Discussion

Growth performance variables includes final BW, ADFI, ADG and FCR. Body weight had a quadratic relationship with  $\alpha$ -tocopherol supplementation on Windsnyer boars. The body weight of pig is an important indicator of its growth, health and readiness to go to the market (Wang *et al.*, 2008). The increase in body weight show that inclusion levels of  $\alpha$ -tocopherol improved growth of Windsnyer boars. The highest body weight was observed on Windsnyer boars supplemented with  $\alpha$ -tocopherol inclusion. The results that show improvement in ADG of Windsnyer pigs when fed inclusion levels of  $\alpha$ -tocopherol are in line with Cheah (1993) who reported significant improvement in the growth rate of Landrace  $\times$  Large White weaner pigs fed  $\alpha$ -tocopherol. As expected, there was a relationship between ADG and  $\alpha$ -tocopherol inclusion level. At low level of  $\alpha$ -tocopherol the ADG increase and high level started decreasing, this suggest the high levels  $\alpha$ -tocopherol are toxic. The increase in ADG could be due to increase in body weight (Whittmore *et al.*, 2003). Cannon *et al.*, (1996) also reported that including vitamin E improves ADG and feed efficiency in pigs. Ugwu and Onyimonyi (2009) reported that dietary deficiency of vitamin E may decrease ADG and could delay growth and development in animals.

The finding that inclusion levels of  $\alpha$ -tocopherol did not influence ADFI a of Windsnyer boars is difficult to explain but suggests that pig consume  $\alpha$ -tocopherol to meet their requirement for growth. Antioxidant vitamins are essential in the animal diet for normal health (Fiego *et al.*, 2004). The observation that inclusion levels of  $\alpha$ -tocopherol did not influence FCR of Windsnyer boars indicates that  $\alpha$ -tocopherol is to great extent, utilizing for meeting growth requirement of pigs.

The testicular development of Windsnyer boars on both left and right testicles were not influenced by  $\alpha$ -tocopherol inclusion level in the diet. The results that showed no influence of  $\alpha$ -tocopherol inclusion levels on testicular development disagrees with Marin-Guzman *et al.* (1997) who reported that length and width of the boar testis were larger to pigs fed into on  $\alpha$ -tocopherol than those fed into diet without  $\alpha$ -tocopherol. The differences between two reports are difficult to explain. It could be that pigs adapted differently to the utilization of the different  $\alpha$ -tocopherol diets. Therefore, it was possible that the supplementation of  $\alpha$ -tocopherol inclusion in the diet did not influence testis weight and epididymis weight of Windsnyer boars.

The observation that histological indexes was not influence by  $\alpha$ -tocopherol inclusion levels in the diet indicates that  $\alpha$ -tocopherol was utilized to maintain the tissue structure and diameter. The seminiferous tubule has high value tendency influence of  $\alpha$ -tocopherol is difficult to explain. The seminiferous tubule average is an important testicular histometric characteristics directly related to testicular weight, tubular diameter and the seminiferous tubules (Valença *et al.*, 2013). The results that showed no influence of  $\alpha$ -tocopherol inclusion to Serti cells this suggests that Serti cells are within normal range and can be used as an accurate index for the evaluation of the efficiency of spermatogenesis. As expected, the density of Leyding cells was numerically wide as inclusion levels of  $\alpha$ -tocopherol increased with histological indexes. But no relationship was observed. The germinal epithelium was not affected by  $\alpha$ -tocopherol inclusion in the diets, this indicate that germinal epithelium are within the normal range. The seminiferous epithelium contained three different germ cell generations namely spermatogonia, spermatocytes and spermatids and one kind of Sertoli cells. This suggests that the thickness of germinal epithelium was determined by the numbers of the spermatogenic and sertoli cells (Garcia-Gil *et al.*, 2002). This relationship could be attributed to difference in specie or supplementation practices  $\alpha$ -tocopherol inclusion levels. The germinal epithelium

values ranged from 87 to 102  $\mu\text{m}$  which are within values stipulated for the pigs (94.4 to 97.7  $\mu\text{m}$ ) (Valença *et al.*, 2013). Consequently, there was no influence of the  $\alpha$ -tocopherol inclusion on testicular development of Windsnyer boars.

### **3.6 Conclusions**

Inclusion of  $\alpha$ -tocopherol caused a quadratic increase in body weight and ADG of Windsnyer boars. These results indicate that  $\alpha$ -tocopherol had a positive role in improving the growth performance. Alpha tocopherol, however, did not influence testicular development and histology.

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## Chapter 4: Inclusion levels of $\alpha$ -tocopherol supplementation on Windsnyer boar spermatozoa characteristics

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### Abstract

The study was conducted to determine the response of supplementing  $\alpha$ -tocopherol on spermatozoa characteristics of Windsnyer boars. Windsnyer boars were fed a diet including 0, 40, 70 and 90 IU of  $\alpha$ -tocopherol. The boars were fed for 18 months. Semen was harvested from the epididymis following euthanization and was assessed using the computer aided sperm analysis software for spermatozoa characteristics. There was no relationship ( $P > 0.05$ ) between  $\alpha$ -tocopherol and semen volume, pH and spermatozoa motility. There was a quadratic relationship between spermatozoa concentration and  $\alpha$ -tocopherol inclusion. As the  $\alpha$ -tocopherol levels increased, the straight-line velocity (VSL) quadratically increased ( $P < 0.05$ ). The equation was  $y = 0.0068x^2 + 0.7679x + 21.983$ . There was a quadratic increase ( $P < 0.05$ ) in live spermatozoa percentage the equation was:  $y = 0.004x^2 + 0.395x + 79.4$ . The proportion of spermatozoa with abnormal head decrease quadratically ( $y = 0.0011x^2 - 0.1064x + 3.5917$ ;  $P < 0.05$ ) with  $\alpha$ -tocopherol inclusion level. In conclusion,  $\alpha$ -tocopherol inclusion had a quadratic response on spermatozoa concentration, live, dead and head abnormal spermatozoa. Alpha tocopherol inclusion was also linear to VSL of Windsnyer boars.

**Key words:** Live spermatozoa percentage, semen quality, straight line velocity, spermatozoa morphology

## 4.1 Introduction

Windsnyer pigs are hardy and can survive and reproduce when fed on fibrous feeds. Their ability to utilise fibrous diets, scavenge for feed resources, which are found in abundance in several production systems is an invaluable attribute that make them suitable for production in resource-poor rural communities. They contribute immensely to household food security, provision of income and other socio-economic functions. Due to the pressure for high production efficiency, the Windsnyer pig population has gradually decreased with the introduction of exotic pig breeds (FAO, 1999). They receive a low priority in pig production endeavours under present agricultural policies. To promote conservation of Windsnyer pigs, the fertility should be improved. Litter sizes for Windsnyer sows averages 7.2 (Chimonyo *et al.*, 2008). It would be highly desirable to increase boar fertility so that litter sizes are only constrained by the number of ova produced. The extent to which fertility of Windsnyer boars can be improved through nutritional manipulation has, however, received little attention.

Vitamin E is an essential nutrient that should be included in pig diets (Umesiobi, 2012) to enhance immunity (Giguere *et al.*, 2002) and metabolism (McDowell, 2002). It is expected to improve spermatogenesis and spermatozoa quality (Wilson *et al.*, 2001) and possibly the fertilization of oocytes. Vitamin E protects cell internal structures against free radicals and is an antioxidant for cellular membrane lipids (Bartle *et al.*, 1980). High concentration of vitamin E in testes and epididymides indicates its importance for the production and maturation of spermatozoa (Kotowska and Kotowski, 2001). Vitamin E deficiencies cause testicular degeneration in chickens, rats hamsters, rabbits guinea pigs and pigs, reducing the number of germ cells and reduction in spermatozoa production (Marin-Guzman *et al.*, 1997). On the range, these pigs are used to scavenging for feed resources. When kept in indoor production

systems, they are subjected to conventional diets. As such, the influence of Vitamin supplementation requires further investigation.

The optimal level of  $\alpha$ -tocopherol needed to improve the function of the reproductive system in boars is highly variable, because of several factors such as the composition of the diet, feed consumption, growth rate and husbandry conditions or stress which exert some influence on conception rate (Flowers, 2002; Umesiobi, 2009). Vitamin E is a lipid-soluble antioxidant (Rutz *et al.*, 2005). An increased dietary intake of  $\alpha$ -tocopherol produces beneficial changes in the antioxidant capacity and lipid profile of semen, maintaining the structural integrity and fertilising capacity of spermatozoa (Surai *et al.*, 1997). Assessing spermatozoa quality includes test of spermatozoa function, as well as evaluation of sperm morphology, motility profiles, concentration, viability and to penetrate oocytes (Tardif *et al.*, 1999). Spermatozoa morphology, spermatozoa concentration and spermatozoa motility are three major components of routine spermatozoa assessment (Söderquist *et al.*, 1991). Masenya *et al.* (2011) argued that spermatozoa motility is an important characteristic in predicting boar fertility. Therefore, the objective of the current study was to determine the response of Windsnyer spermatozoa characteristics to  $\alpha$ -tocopherol supplementation in the diet. It was hypothesized that  $\alpha$ -tocopherol inclusion influences Windsnyer spermatozoa characteristics.

## **4.2 Material and methods**

### **4.2.1 Study site**

Details on the description of the study site are given in Section 3.2.1.

### **4.2.2 Pigs, treatments and experimental design**

The experimental diets and experimental was described in section 3.2.2.

#### **4.2.3 Management of pigs, slaughtering procedure and semen collection**

After 18 month-of growth experiment, boars were slaughtered at the age of 21 months weighing  $57.2 \pm 1.67$  (mean  $\pm$  standard deviation) Slaughtering was done at the Meat Science Abattoir at Agriculture Research Council. After electric stunning, a sharp knife was then used to cut the anterior vena cava. While the pig was bleeding, testis and epididymides were removed before the pig was dipped into hot water and trimmed of adhering tissue. Spermatozoa samples were collected from epididymis through making incision with a razor. Epididymal semen was collected directly into 15 ml tubes and immediately placed into an insulated thermoflask at 37°C. The collected semen was then transported to the laboratory for macroscopic and microscopic spermatozoa evaluation within one hour.

#### **4.2.4 Determination of spermatozoa characteristics**

The pH of the semen was determined using a litmus paper (Med-Test Combi 9™). The semen sample was dropped on the paper and pH was read. The volume of epididymal semen was measured when it was extracted down into the graduated 15ml tube. Semen that was not pure white in colour was discarded, as it considered to have been contaminated by blood during extraction. Spermatozoa concentration was determined using a spectrophotometer. A 3ml of a 2.9 % sodium citrate solution (pH 7.0) in a square cuvette was placed inside the spectrophotometer in the first four slots of the cuvette holder. The cuvette was removed 40 $\mu$ l of epididymal semen was added, before the mixture. The cuvette was then placed in the spectrophotometer to measure absorbance. Spermatozoa concentration was calculated using the formular: Porcine (dilution factor (76)) x (21.39 x absorbance)-1.09).

#### **4.2.5 Spermatozoa motility**

The Computer Assist Sperm Class Analysis (CASA) system was used to assess spermatozoa motility with the aid of a Sperm Class Analyzer® -(SCA®) (V.5.2 Animal/Veterinary Microptic S.L, Barcelona, Spain). For the spermatozoa swim-up technique, 10µl of epididymal semen was diluted with 500µl of Beltsville Thawing Solution (BTS) medium and placed in the incubator for 5 minutes. Following incubation, 5µl of the semen sample was placed on a microscopic glass slide cover with a microscopic cover slip and examined under x10 magnification with SCA® microscopic projecting an image on a monitor. Motility characteristics evaluated were expressed as the percentage progressively motility spermatozoa (PM) (spermatozoa with forward movement or swimming in a most straight line) and percentage non-progressively motility spermatozoa (NPM) (spermatozoa that move but don't make forward progression or swim in very tight circles. Spermatozoa velocity characteristics evaluated included rapid (spermatozoa moving quickly), medium, slow, curvilinear velocity speed (VCL)(the average path velocity of the sperm head along its actual trajectory), straight line velocity (VSL) (the average path velocity of the sperm head along a straight line from its first to its last position), average path velocity (VAP) (the average velocity of sperm head along its average trajectory), linearity (LIN) (the ratio between VSL and VCL), straightness (STR) (the ratio between VSL and VAP) and wobble (WOB).

#### **4.2.6 Spermatozoa morphology**

Spermatozoa morphology was evaluated using eosin nigrosin stain. Briefly, 7µL of boar semen was added to 20µL of eosin nigrosin staining solution in a 0.6mL micro-centrifuge graduated tube and mixed gently. A drop of 5µL boar semen and eosin nigrosin stain was placed on a clear end of a microscope slide and smeared. Fluorescent microscope was used at 100x magnification to count 200 spermatozoa per each stained-glass slide. The spermatozoa that had

absorbed the dye (appearing red) was considered as percentage of dead spermatozoa and those that did not absorb the dye were alive. The abnormal spermatozoa head (flat, sharp, double and if is not oval), mid piece (proximal and distal) and tail (coiled, double and broken) were also counted.

### 4.3 Statistical analyses

A polynomial regression (PROC REG) procedure of (SAS, 2008) was used to determine the relationships between  $\alpha$ -tocopherol inclusion and spermatozoa characteristics. The model that was used was:

$$Y = B_0 + B_1A + B_1A^2 + E$$

Y = is the response variable (semen motility, velocity and sperm morphology)

$B_0$  = is the intercept

$B_1A$  = linear regression component

$B_1A^2$  = quadratic regression component

E = is the error

## 4.4 Results

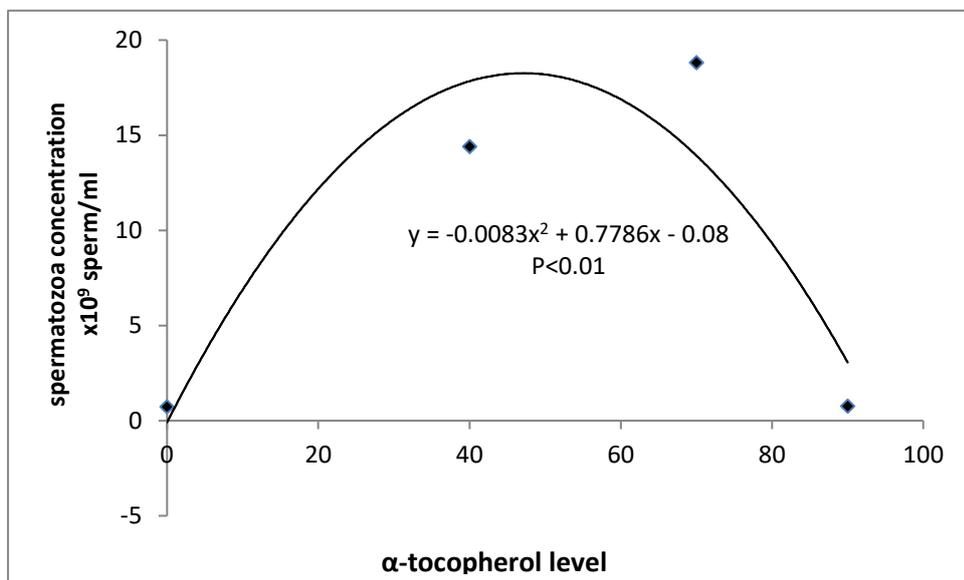
### 4.4.1 Macroscopic spermatozoa characteristics

Table 4.1 indicates the response of inclusion levels of  $\alpha$ -tocopherol on spermatozoa macroscopic characteristics of Windsnyer boars. There was no relationship ( $P > 0.05$ ) of supplementation of  $\alpha$ -tocopherol to the diet on pH and semen volume. Figure 4.1 shows the relationship between  $\alpha$ -tocopherol supplementation on spermatozoa concentration. There was a quadratic increase on spermatozoa concentration ( $P < 0.05$ ). Inclusion levels of  $\alpha$ -tocopherol increased spermatozoa concentration  $10^9$  spermatozoa/ml. As the level of  $\alpha$ -tocopherol inclusion level increased, there was quadratic relationship on spermatozoa concentration. There

was a quadratic increase in spermatozoa concentration of pigs when fed increasing levels of  $\alpha$ -tocopherol level was ( $y=0.0083x^2 + 0.778x - 0.08$ ;  $R^2= 0.84$ ;  $P<0.05$ ).

**Table 4.1: Effect of  $\alpha$ -tocopherol inclusion levels on macroscopic semen characteristics of Windsnyer boars**

	Different level of $\alpha$ -tocopherol (IU)					P-value	
	0	40	70	90	SEM	Linear	Quadratic
<b>Semen volume (ml)</b>	5.1	8.9	7.6	5.1	0.10	0.72	0.14
<b>Semen pH</b>	7.0	7.0	7.0	7.0	0.00	0.63	0.84



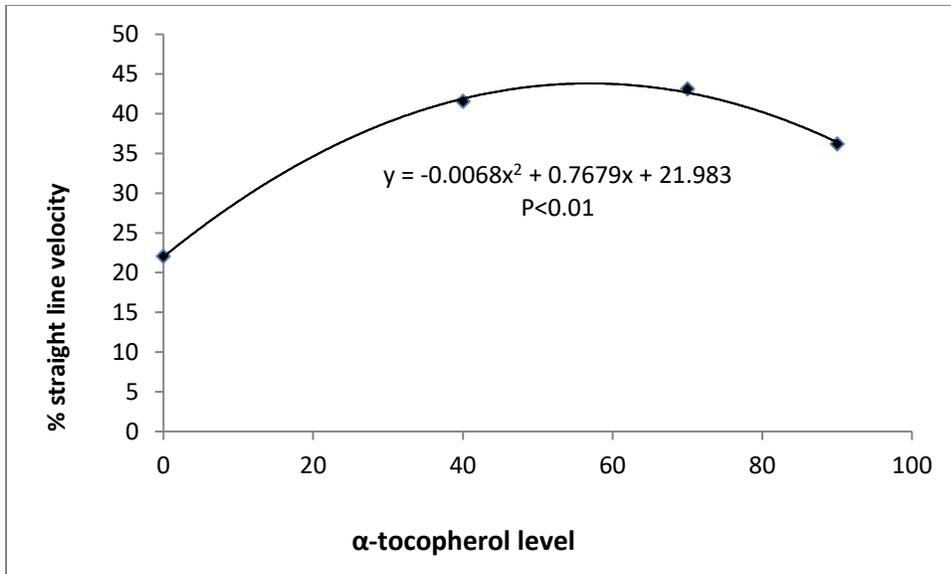
**Figure 4.1: Relationship between α-tocopherol and spermatozoa concentration**

#### **4.4.2 Spermatozoa motility characteristics**

The response of inclusion levels of  $\alpha$ -tocopherol on Windsnyer boar sperm motility is indicated in Table 4.2. There was no relationship on some spermatozoa motility and  $\alpha$ -tocopherol inclusion level ( $P > 0.05$ ). There was a quadratic relationship between the straight line (VSL) and different level of  $\alpha$ -tocopherol. The relationship between inclusion level of  $\alpha$ -tocopherol and VSL is illustrated in Figure 4.2. As the  $\alpha$ -tocopherol levels increased, there was a quadratic decrease in VSL characteristics. The regression equation between VSL and  $\alpha$ -tocopherol level was ( $y = -0.0068x^2 + 0.7679x + 21.983$ ;  $R^2 = 0.99$ ).

**Table 4.2: Relationship between inclusion levels of  $\alpha$ -tocopherol and spermatozoa motility characteristics of Windsnyer boars**

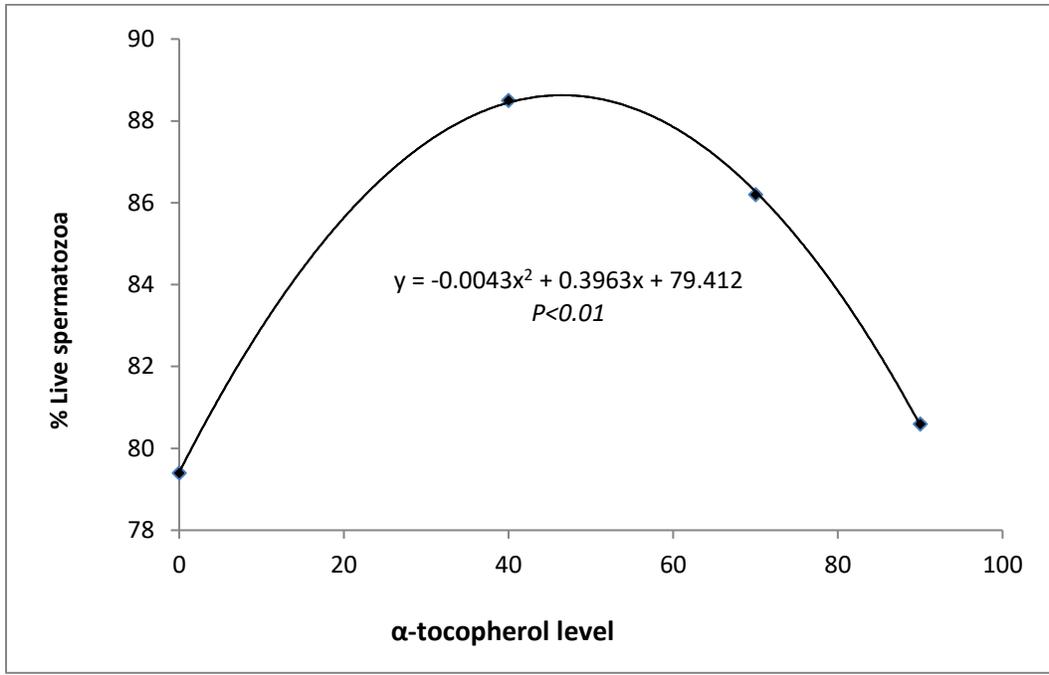
<b>Component</b>	<b><math>\alpha</math>-Tocopherol Inclusion (IU)</b>					<b>SEM</b>	<b>P VALUE</b>	
	<b>0</b>	<b>40</b>	<b>70</b>	<b>90</b>	<b>Linear</b>		<b>Quadratic</b>	
Total motility (%)	88.5	91.2	88.5	88.2	1.6	0.760	0.269	
Progressive motility (%)	39.6	37.3	40.7	43.7	7.4	0.538	0.510	
Non-progressive motility (%)	48.8	53.9	47.8	43.5	5.56	0.451	0.309	
Rapid (%)	31.3	41.2	39.4	33.9	5.82	0.642	0.204	
Slow (%)	20.4	15.2	18.9	16.9	3.43	0.597	0.590	
Medium (%)	37.7	34.8	31.9	27.4	5.99	0.211	0.763	
Curvilinear velocity ( $\mu\text{m}/\text{Sec}$ )	145.7	131.1	144.1	143.0	7.63	0.976	0.309	
Average path velocity ( $\mu\text{m}/\text{Sec}$ )	65.3	74.2	69.9	75.6	9.02	0.480	0.874	
Wobble (%)	57.0	57.1	56.7	56.3	4.36	0.811	0.950	
Straightness (%)	65.9	57.5	56.7	56.9	5.47	0.2034	0.517	



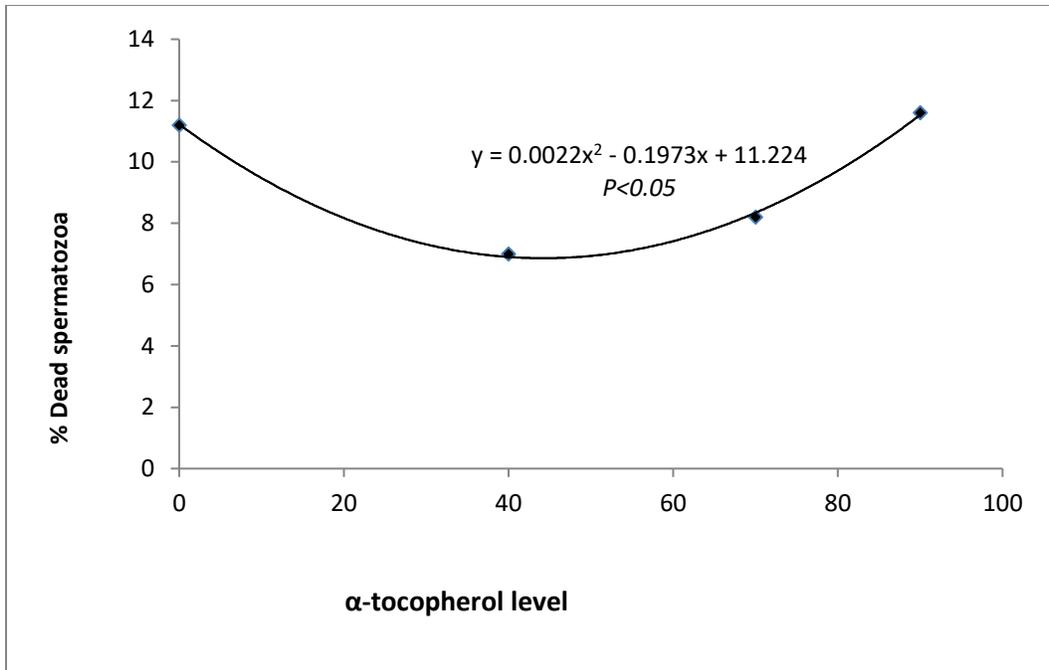
**Figure 4.2: Response of  $\alpha$ -tocopherol on straight-line velocity spermatozoa of Windsnyer boars**

### 4.4.3 Spermatozoa morphology

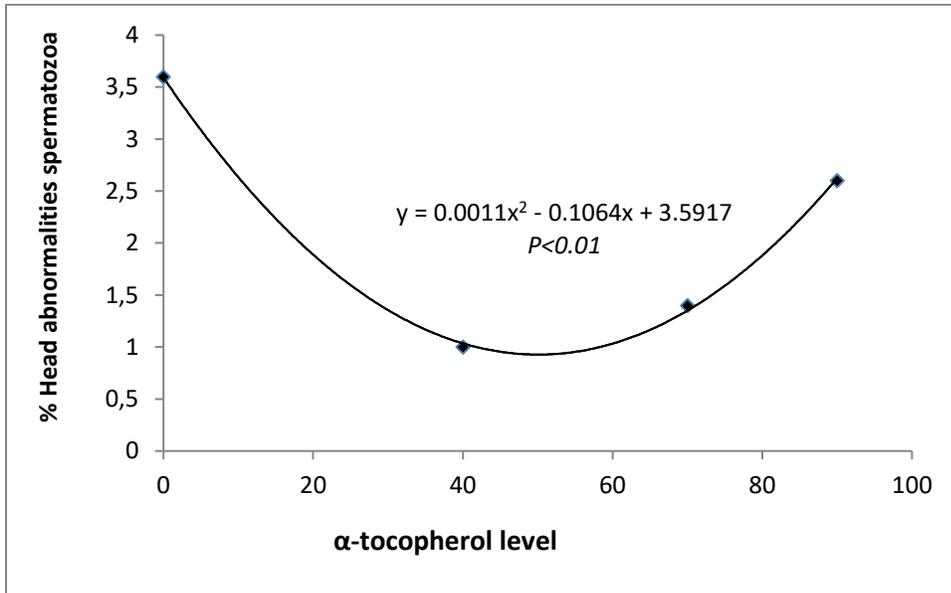
The relationship between  $\alpha$ -tocopherol supplementation and percentage of live spermatozoa of Windsnyer boars is indicated Figure 4.3. Live percentage of spermatozoa increased quadratically with supplementation of  $\alpha$ -tocopherol inclusion level ( $P < 0.001$ ). Inclusion of  $\alpha$ -tocopherol at 40IU and 70IU resulted increasing live spermatozoa ( $P < 0.05$ ). The regression equation for live spermatozoa percentage was  $y = -0.0043x^2 + 0.396x + 79.412$ . Inclusion levels of  $\alpha$ -tocopherol quadratic significantly increase ( $P < 0.05$ ) spermatozoa viability. Figure 4.4 shows the relationship between  $\alpha$ -tocopherol inclusion and dead spermatozoa. There was quadratic relationship of dead spermatozoa and  $\alpha$ -tocopherol inclusion to Windsnyer boars. The level of  $\alpha$ -tocopherol inclusion level decreased ( $P < 0.05$ ) with increase in  $\alpha$ -tocopherol level ( $y = 0.0022x^2 - 0.1973x + 11.224$ ). Figure 4.5 shows the relationship between percentage of abnormal head and  $\alpha$ -tocopherol. There was a quadratic relationship between  $\alpha$ -tocopherol and head abnormalities. The proportion of spermatozoa with abnormal head decrease ( $y = 0.0011x^2 - 0.1064x + 3.5917$ ;  $P < 0.05$ ). There was no relationship between mid-piece and coiled tail of Windsnyer boars and  $\alpha$ -tocopherol inclusion levels ( $P > 0.05$ ). Both mid-piece and coiled tail abnormalities of spermatozoa were not affected by  $\alpha$ -tocopherol inclusion.



**Figure 4.3: Relationship between  $\alpha$ -tocopherol and live spermatozoa percentage**



**Figure 4.4: Response of  $\alpha$ -tocopherol inclusion and dead spermatozoa percentage**



**Figure 4.5: Relationship between α-tocopherol and percentage head abnormalities spermatozoa**

#### 4.5 Discussion

Alpha-tocopherol inclusion level supplemented to Windsnyer boars had no influence some spermatozoa characteristics but these characteristics were within the normal range. Although it was hypothesised that  $\alpha$ -tocopherol inclusion influence Windsnyer spermatozoa characteristics. The observation that semen pH and volume was not influenced by  $\alpha$ -tocopherol inclusion is difficult to explain. Epididymis spermatozoa volume in the current study was very low due to methods of collection. Kondracki, (2003) reported that the standard ejaculate semen volume of 150 to 300ml in Large White pigs. King and MacPherson, (2005), reported that the semen pH of the boar sperm fraction was  $7.69 \pm 0.33$ . Increase  $\alpha$ -tocopherol inclusion level is expected to produce a quadratic relationship with semen concentration of between  $0.7$  and  $1.2 \times 10^9$  spermatozoa/ml. Similarity, Turba *et al.* (2007), reported that boar semen consists of a dense ejaculate which contains between  $0.2$  and  $0.4 \times 10^9$  spermatozoa/ml. Brzezinska-Slebodzinska *et al.* (1995) observed that supplementation with vitamin E increased the concentration of spermatozoa in semen, a possibly linked to the antioxidant properties of this vitamin.

The spermatozoa motility was not influenced by  $\alpha$ -tocopherol inclusion in the diet of is difficult to explain but indicates that  $\alpha$ -tocopherol are great extent, utilizing for meeting normal range spermatozoa motility. Yi *et al.* (2008) reported that the sperm-rich fractions of ejaculates with  $>85$  % motile sperm. Umesiobi, (2012) showed in a positive relationship of  $\alpha$ -tocopherol supplementation on spermatozoa characteristics. Selvan, (2007) observed no relationship on semen quality characteristics when breeder boars were supplemented  $\alpha$ -tocopherol. (10 versus 40 IU/kg diet). The observation that  $\alpha$ -tocopherol inclusion is linear related to straight line velocity was not expected but this indicates  $\alpha$ -tocopherol has ability to improve the rate of spermatozoa to

swam faster. The relationship of VSL and  $\alpha$ -tocopherol based on this finding with spermatozoa epididymis, is predictive of fertility. Froman *et al.* (1999) reported that the significant of VSL on spermatozoa is the predictive of fertility. The response velocity characteristics indicate active spermatozoa metabolism and are a great importance for fertilizing to take place. This suggest that the spermatozoa will be more efficient at traveling through the reproductive tract and reaching the site of sperm storage or fertilization. King *et al.*, (2000) reported that straight line velocity and linearity is important determinants in motility phenotypes and may influence the ability of sperm to traverse the female reproductive tract, there by affecting subsequent fertility.

The observed quadratic relationship between  $\alpha$ -tocopherol and live spermatozoa percentage suggests an increase in  $\alpha$ -tocopherol could ensure high percentage of viable spermatozoa and improve quality of spermatozoa. Dietary antioxidants prevent oxidative damage to spermatozoa by scavengers of free radicals and increase viable spermatozoa. Similarly, Marin-Guzman *et al.* (2000) reported that high levels of dietary  $\alpha$ -tocopherol supplementation prevented the spermatozoa from changing in morphological structure, possibly because  $\alpha$ -tocopherol acts as an intracellular antioxidant (McDowell, 2002).

Umesiobi, (2012) reported boars supplemented with a diet containing  $\alpha$ -tocopherol had relationship spermatozoa viability. Salisbury and VanDemark, (1961) suggested the relationship between sperm quality and quantity, when fertility increases with increasing number of live spermatozoa. Therefore, the antioxidant is crucial element in boar semen quality and  $\alpha$ -tocopherol supplementation has unique role via antioxidant mechanisms to protect the spermatozoa from free radicals. Marin-Guzman, (2000) reported that the addition of  $\alpha$ -tocopherol had a little relationship

to morphological changes in spermatozoa or other parameters. The observation that  $\alpha$ -tocopherol inclusion is quadratically related to dead and abnormalities spermatozoa was expected. The observed mid-piece and coiled tail spermatozoa were not influenced by  $\alpha$ -tocopherol inclusion this could be explained by the ability of  $\alpha$ -tocopherol to scavenge the free radicals from damaging spermatozoa. Didion (2008) suggest that when more than 30% abnormal spermatozoa in the semen are abnormal, fertility is reduced.

#### **4.6 Conclusions**

Inclusion level of  $\alpha$ -tocopherol quadratically increased the spermatozoa concentration, straight line velocity and live percentage spermatozoa. The dead and abnormal head spermatozoa decreased quadratically with  $\alpha$ -tocopherol inclusion. Inclusion level  $\alpha$ -tocopherol influenced the semen quality in a quadratic manner. Spermatozoa motility was, however, not influenced by  $\alpha$ -tocopherol inclusion.

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### 5.1 General Discussion

The hypothesis of this dissertation was to assess influence of  $\alpha$ -tocopherol inclusion level and semen fertility of Windsnyer boars to make recommendation to improve fertility. Utilisation of Windsnyer pigs has potential to increase food security, reduce poverty and improve livelihoods of farmers. The slow growth and decline in fertility of Windsnyer pigs is a major concern in conservation of this breed. Dietary supplementation of  $\alpha$ -tocopherol inclusion on growth and semen fertility of the Windsnyer pigs to make recommendations to maximise fertility.

Response to growth performance and testicular development of Windsnyer boar  $\alpha$ -tocopherol supplementation in Chapter 3. The hypothesis was that  $\alpha$ -tocopherol inclusion influences growth performance and testicular development of Windsnyer boars. The observation that ADFI and FCR were not influenced by  $\alpha$ -tocopherol inclusion levels that  $\alpha$ -tocopherol is great extent, utilizing for meeting the growth requirement of pigs. As expected, there was a significant response of  $\alpha$ -tocopherol in body weight and ADG. Whittemore *et al.* (2003) reported that observed quadratic relationship of ADG and  $\alpha$ -tocopherol could be due to increase in body weight, adaptation to feed. Testicular development was not influenced by  $\alpha$ -tocopherol inclusion level in the diet supplemented to Windsnyer boars. The histology index was not related to  $\alpha$ -tocopherol inclusion level but the indexes remain at the normal range. There was a tendency of  $\alpha$ -tocopherol inclusion level to affect seminiferous tubules in Windsnyer boars. Alpha-tocopherol deficiencies caused testicular degeneration in pigs and resulted in a lower number of germ cells and reduction in sperm production (Cooper *et al.*, 1987).

In Chapter 4, the study was determined whether response of Windsnyer spermatozoa characteristics to  $\alpha$ -tocopherol inclusion. The hypothesis tested was that  $\alpha$ -tocopherol influence Windsnyer boar's spermatozoa characteristics. It was observed that semen volume and semen pH was not influenced by  $\alpha$ -tocopherol inclusion levels in the diet of Windsnyer boars ( $P>0.05$ ). The observation that  $\alpha$ -tocopherol inclusion was quadratically related to spermatozoa concentration was expected, suggesting that inclusion level of  $\alpha$ -tocopherol supplementation to Windsnyer boars was effective. Despite reported benefits of supplementation with  $\alpha$ -tocopherol, inclusion had no influence on spermatozoa motility. There was a significant response in straight line velocity (VSL) to  $\alpha$ -tocopherol inclusion. Morphological evaluation of epididymal spermatozoa of boars demonstrated that supplementation of  $\alpha$ -tocopherol has positive relationship with live spermatozoa. Jones and Mann (1977) demonstrated that peroxidative structural damage to spermatozoa occurred when unsaturated fatty acids were present in semen, resulting in a decline in spermatozoa motility. The percentage of dead and head abnormalities spermatozoa was low in boars not supplemented with Vitamin E, indicating that dietary supplementation of  $\alpha$ -tocopherol decreased the occurrence of abnormal spermatozoa. Alpha-tocopherol may serve as an antioxidant, thereby neutralising free radicals and prevents oxidation within the membrane in boar semen.

## **5.2 Conclusions**

Dietary supplementation of  $\alpha$ -tocopherol inclusion level has a positive role in improving the body weight and ADG of testis in Windsnyer boars. The supplementation of  $\alpha$ -tocopherol inclusion levels influenced spermatozoa concentration, straight line velocity and spermatozoa morphology. Supplementation of  $\alpha$ -tocopherol inclusion level to Windsnyer boars improved semen quality.

Dietary  $\alpha$ -tocopherol supplementation decreased the incidence of dead and abnormal spermatozoa which may improve the fertilising capability of the spermatozoa.

### **5.3 Recommendations**

This study shows that supplementation of  $\alpha$ -tocopherol inclusion level increase ADG, sperm concentration, live percentage spermatozoa and VSL. Alpha tocopherol inclusion decrease the dead and head abnormal spermatozoa. Future studies need to be done to:

- Identify the accurate optimum inclusion level of  $\alpha$ -tocopherol.
- Understand the mechanism in which  $\alpha$ -tocopherol improve average daily gain.
- Understand the effectiveness of  $\alpha$ -tocopherol to libido of boars.
- Evaluate the physiological effect of  $\alpha$ -tocopherol on spermatozoa motility.
- Assess molecular reason for the influence of  $\alpha$ -tocopherol, particularly in live spermatozoa check damage membrane.
- Understand effect of  $\alpha$ -tocopherol on different breeds.

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