EFFECTS OF DIETARY SELENIUM SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF SOWS

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TITLE PAGE

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A PROJECT WORK SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc.) OF THE UNIVERSITY OF NIGERIA, NSUKKA

DEPARTMENT OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
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DECEMBER, 2014

CERTIFICATION

We certify that this project work was carried out by **AMAEFULE, BRIGHT CHIGOZIE (Reg. No: PG/M.Sc./12/62185)** and approved by the Department of Animal Science, University of Nigeria, Nsukka.

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EXTERNAL EXAMINER

DATEé é é é é é é é .

DEDICATION

Dedicated to God whose abundant mercy and grace kept me all these years and in loving memory of Igwe, Darlington. (R.I.P).

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ABSTRACT

This study was conducted to investigate the effect of dietary selenium (se) supplementation on the reproductive performance of sows, preweaning growth performance of their piglets, haematological and antioxidant status of pigs. Twelve (12) crossbred sows (Landrace × Large White) aged 1-2 years with an average body weight (BW) of 56.1 ± 5kg were randomly allotted to four treatment groups of three sows per treatment in a completely randomised design (CRD). The treatment groups comprised T_0 (no Se supplementation), and T_1, T_2, T_3 administered with 0.30 mg/Kg selenium-methionine during second trimester of gestation, third trimester of gestation and lactation respectively. Selenium supplementation significantly ($P<0.05$) improved reproductive efficiency as measured by larger litter size (T_0; 5.67 ± 0.33, T_1; 6.00 ± 92.47, T_2; 7.66 ± 1.20, T_3; 8.33 ± 8.33) reduced mortality,(T_0; 2.33 ± 0.33%, T_1; 1.00 ± 0.49%, T_2; 0.66 ± 0.33%, T_3; 0.47 ± 3.05%), and increased litter weight at weaning (T_0; 5.07 ± 5.92 kg, T_1; 5.79 ± 0.02kg, T_2; 5.76 ± 2.64kg, T_3; 5.93 ± 1.85kg), in the Selenium treated groups when compared to the control group. Haematological parameters were not significant ($P>0.05$) among treatments during the second trimester of gestation. However, Se supplementation significantly ($P<0.05$) increased Pack Cell Volume (PCV) (T_0; 32.50 ± 0.25%, T_1; 38.50 ± 0.08%, T_2; 37.00 ± 1.00%, T_3; 34.50 ± 0.50%) and Red Blood Cell (RBC) (T_0; 131.50 ± 28.5, T_1; 185.0 ± 35.0, T_2; 177.5 ± 32.5, T_3; 210.0 ± 20.0) during the third trimester and Red Blood Cell (RBC) (T_0; 108.0 ± 2.00, T_1; 165.0± 15.0, T_2; 175.0 ± 5.00, T_3; 165.0± 5.00) and White Blood Cell (WBC) (T_0; 22.07±0.44, T_1; 11.20±0.80, T_2; 9.00± 1.00, T_3; 10.00±3.60) during lactation. All Se supplemented groups showed significantly increased plasma Selenium concentration in all stages compared with the control. Dietary selenium supplementation significantly increased plasma Glutathione ï S- Reductase, Glutathione peroxidase (GSH-Px) activity during lactation (T_3) and significantly ($P<0.05$) decreased plasma Malondialdehyde (MDA) (T_0; 3.76 ± 1.74, T_1; 0.12 ± 0.03, T_2; 0.11±0.35, T_3; 4.36 ± 0.62), (T_0; 7.26 ± 0.08, T_1; 3.60 ± 0.22, T_2; 3.70 ± 0.27, T_3; 2.27 ± 0.11),content in the third tremester of gestation and Lactation respectively. Superoxide Dismutase (SOD) and catalase activity in all treatment groups was significantly ($P<0.05$) different compared to the control. From these results, it was concluded that dietary selenium supplementation increased reproductive efficiency and enhanced haematological and serum anti-oxidant enzyme activities of pigs especially during gestation and lactation.

CHAPTER ONE

1.0 INTRODUCTION

Food security refers to the availability of food and oneôs access to it and it exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (WHO, 1996). As human population continues to grow, with the greatest growth expected in countries that are already suffering from chronic hunger and malnutrition, there will be need to ensure food safety for all and especially the more susceptible sector of human population (Adeola and Olukori, 2009). It is a well known fact that the growth rate of human population far exceeds that of animal protein supply and this great disparity creates a problem for food security. Consequently, the demand for animal protein exceeds the supply (Ikani and Dewfang, 2004).

The present average daily protein intake among Nigerians is 45.5g per head per day as against the FAOôs recommended minimum intake of 62g per head per day (FAO, 1996). Similarly, the International Conference on Nutrition (ICN) reported that low income rural and semi urban adult dweller in Nigeria consumed less than 60% of their caloric need (which is 2400Kcal/head/day) and less than 40% of their protein needs (ICN, 1992). It seems apparent that animal protein intake of average Nigerian has not yet improved over the years irrespective of government policies towards achieving food security in Nigeria. This therefore suggests more radical approach to the problem.

Pig production which is recently on the increase is part of the efforts and one of the fastest means of bridging the prevailing animal protein deficiency gap which has developed over the years due to increasing population (Serres, 1992). Eusebio, (1980) described the pig as one of the most prolific and fast growing livestock that can convert food waste to valuable product. Their annual growth rate (3.8%) is higher than that of the human population (2.3-

2.8%) (Shaib *et al.*, 1997). Pigs excel other animals, such as cattle, sheep and goats in converting feed to flesh (Ikani and Defwang, 1995). Pigs have unique potentials as possible solutions to Nigerian meat supply situation because of their high fertility rate, short generation interval, high litter size and growth rate, high efficient carcass yield and easy adaptation to environmental conditions (Balogun, 1981, Adekunle, 1995, Ugwu *et al.,* 1997). Despite these attributes, productivity of pigs in Nigeria has been low due to the problems of poor nutrition caused by inadequate, excess or imbalanced nutrient intake, high cost of feedstuff, poor health and management and the effect of the changing climatic conditions alongside its heavy thermal load on the animals which adversely have affected the various stages of the reproductive event (Machebe *et al.,* 2009). These events include delayed puberty, reduced ovulation and lower conception rates, high embryonic and foetal losses, excessively long post-partum anoestrus, poor lactation, high prenatal mortality and poor neonatal performance etc. (Martin, 2012)

Trace minerals/ micronutrients like Zinc, Selenium, Vitamin A, Vitamin E, Copper and Molybdenum have been known to reduce or ameliorate these reproductive challenges in animals. For instance, Vitamin E functions as an intra-cellular antioxidant scavenging for free reactive oxygen and lipid hydroperoxides, and converting them to non-reactive forms, thus maintaining the integrity of membrane phospholipids against oxidative damage and peroxidation (Surai, 1999). Vitamin A is known to reduce delayed puberty, low conception rate, high embryonic mortality and improve libido (Allan *et at.,* 1999, Smith and Somade, 1994). Zinc on the other hand reduces impaired spermatogenesis and aid development of secondary sex organs in males, improves fertility and litter size in multiparous species (Underwood and Suttle 2003). Copper is involved in steriodogenesis and prostaglandins secretion which in turn improves fertility, prevents delayed/depressed oestrus, and abortion/foetal resorption (Smith and Akinbamijo 2000).

Selenium (Se) is an essential element playing an important role in animal reproduction (Surai, 2002). It can be found in the body parts as a part of at least 25 selenoproteins. These selenoproteins are considered to be involved in the regulation of various physiological functions including anti-oxidant protection, regulation of gene expression, thyroid metabolism, immune response, reduction of inflammation and maintenance of sperm structure integrity (Surai, 2002, Arthur and Geoffrey 2003 and Schomburg *et al.,* 2004). There are two main sources of selenium in the diet: organic selenium (mainly selenomethionine) and inorganic selenium (selenite and selenate). Surai (2002) indicated that the organic form of selenium provides more selenium reserves in the body and a more efficient transfer to the egg in poultry and milk in lactating animals.

Feeding selenium to male farm animals have shown that selenium seems to be essential for normal spermatozoa development but little attention has been devoted to female reproductive performance.

1.1 Objectives of the study

The broad objective of this study was to evaluate the effect of dietary selenium supplementation on the reproductive performance of sows.

1.1.2 Specific Objectives

1. To determine the effect of dietary selenium supplementation on the reproductive performance of sows.
2. To determine the effect of selenium supplementation on pre-weaning growth performance of piglets.
3. To evaluate the effect of dietary selenium supplementation on the haematological and oxidative enzyme status of sows.

1.2 Justification of the Study

Pigs have unique potentials as possible solutions to Nigerian meat supply situation because of some attributes like their high fertility rate, short generation interval, high litter size and growth rate, high efficient carcass yield etc. (Balogun, 1981, Adekule, 1995, Ugwu *et al.,* 1997). Despite these attributes, productivity of pigs in Nigeria has been low due to the problems of poor nutrition, poor health and management. The effect of the changing climatic conditions also imposes heavy thermal load on the animals with the pregnant and lactating animals at the greatest risk of being negatively affected because of their high performance level (growth of pregnancy and lactation) (Hahn, 1995).

Regarding selenium and female fertility only sparse information exists and little attention has been devoted to female reproductive performance, and the data are insufficient for drawing appropriate conclusion on its importance.

It therefore becomes justifiable to design a study to determine the impact and the best stage of gestation (second or third trimester) or during lactation for dietary selenium supplementation that will improve the reproductive performance of sows and growth of their piglets without any negative or detrimental effect on the physiology of the animals.

It is believed that the findings of this research if made available to farmers will help farmers appropriate decision on the effectiveness or otherwise of selenium inclusion as dietary supplement in pig diet to improve the reproductive potentials of the animals especially in the tropics where climatic and other environmental variables (nutrition, disease) have hindered pig productivity. It is expected that on adoption of the finding of this research by farmers, pig productivity will improve leading to an adequate supply of animal protein to consumers. It will also lead to the sustainability of food security situation in Nigeria and other developing economies of the world.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Selenium Metabolism

2.1.1 What is Selenium

Selenium (Se) is an essential mineral and microelement in the diets which is required for maintenance of health, growth and biochemical- physiological functions in humans and animals such as biological antioxidant, immune function, reproduction and thyroid hormone metabolism (Surai, 2006) . The discovery of selenium, as an element, was made in 1817 by the Swedish chemist, Jöns Jakob Berzelius, through what was, at that time, an elegant analytical process (Oldfield, 2006). While Berzelius was the first to isolate and chemically characterize Se, he was probably not its original discoverer. That achievement should, perhaps, be attributed to the 13th century Italian scholar, Arnold of Villanova, who described the Se in his book *Rosarium Philosophorum*. Selenium is a two-faced element.

Selenium was identified from the Greek word ΣελήνηΣ (SelŮnŮ), which signifies the moon (Reilly, 2006). Like the moon, after which it is named, it has both a dark and a bright side. This duality has, right from the time the element was first isolated, presented science with a dilemma: how to reconcile its apparently contradictory properties and roles. In the early thirteenth century, the Venetian traveller Marco Polo, journeying through western China, observed that in one region, merchants prevented their pack animals from eating certain local plants, it has been argued by Reilly 2004). Since its discovery, Se has had an interesting history. In biology, it was initially known to be toxic to livestock when there were indications of Selenium as carcinogen and later Se shown to be essential micronutrient with anti-carcinogenic properties (Sunde, 1997; Oldfield, 2006 and Reilly, 2006).

2.1.2. The Chemistry of Selenium

Selenium, atomic number 34 on the periodic table of elements, is a member of Group 16 (Previously Group VIA) along with oxygen, sulphur, tellurium and polonium (Sunde, 1997). Commonly Se exists in four valence states and the ï 2 state predominates in organic Se compounds (Kabata-Pendias and Mukherjee, 2007). It is classified as a metalloid element, indicating that it has both metallic and non-metallic properties. Its natural atomic weight is 78.96 and Se has six naturally occurring stable isotopes with the 78Se and 80Se forms accounting for over 73% of the total isotopes. (Payne, 2004 and Reilly, 2004). Like most non-metals, Se can exist in both positive and negative oxidation states (Halka and Nordstrom, 2010). Common oxidative states are present as the following in Table (1) according to Brigelius-Flohe *et al.* (2002).

Table 1: Oxidation states of selenium.

Chemical structure	Oxidation states	Name of acid	Name of salt
H2Se	ī 2	Hydrogen selenide	Selenide
R-she	ī 2	Selenol	
Se	0	Selenium	
R-Se-OH	0	Selenenic acid	Selenenate
O			
II			
R-Se-OH	+2	Seleninic acid	Seleninate
O			
II			
HO-Se-OH	+4	Selenious acid	Selenite ($SeO3^{2-}$)
O			
II			
HO-Se-OH	+6	Selenic acid	Selenate ($SeO4^{2-}$)
II			
O			

Source: Brigelius-Flohe *et al.* (2002)

2.1.3. Selenium Metabolism and Absorption

The metabolism of Se is dependent on its chemical form and on the amount ingested. However, the location in the gastrointestinal tract where Selenium is absorbed, regardless of Se source, seems to be consistent. Wright and Bell (1966), using sheep and pigs and Whanger *et al.* (1976), using rats, agree that the majority of dietary Se is absorbed in the duodenum. Whanger *et al.* (1976) also indicated that there was some absorption of Se in the jejunum and ileum but practically none from the stomach. However, there does seem to be differences in type of absorption depending on source. Combs and Combs (1986) indicated that inorganic sources of Se, such as inorganic sodium selenite or selenates, are passively absorbed, while organic sources, such as Se-enriched yeast or selenomethionine, are actively absorbed via amino acid transport mechanisms, whereas selenite is absorbed by simple diffusion, and selenate by a sodium-mediated carrier shared with sulfate.

No homeostatic control of Se absorption has been identified or presumably exists as dietary Se levels or body Se status has no apparent impact on its absorption efficiency. However, Se absorption does vary, depending on its chemical form, the animal species, and a number of dietary factors. Organic Se, such as selenomethionine or Se enriched wheat and yeast, has a greater absorption rate than inorganic Se in many species, resulting in lower excretion of Se in the faeces. Selenate is better absorbed than selenite, and both are better absorbed than elemental Se. Due to the reduction of selenite and selentate, and the formation of insoluble particles in the rumen, cattle and sheep have lower absorption of Se with greater variations than non ruminant species (Wright and Bell, 1966). Many sources of Se are readily absorbed in the small intestine by animals and humans, and regulation of body Se retention may occur via urinary excretion (NRC, 2005).

According to Axley and Stadtman, 1989 selenate first is converted to selenite. Then, selenite is non-enzymically reduced to elemental Se by glutathione forming seleno-

diglutathione (GS-Se-SG) (Ganther, 1966). In the absence of oxygen, seleno-diglutathione is further reduced to selenide (HSe-) by glutathione reductase (Hsieh and Ganther, 1975). At this point, selenide can have several different fates. It can be methylated to form methaneselenol (CH3SeH), which then can form dimethylselenide or trimethylselenonium ion (CH3)XSeH) (Hsieh and Ganther, 1977). Selenide also can bind to the Selenium binding proteins, or it can be a substrate for selenophosphatesynthetase for the tRNA-mediated synthesis of selenoproteins (Sunde, 1997). This last step converts inorganic Se into the organic forms of Se that are found in mammalian tissues. Organic Se is metabolized differently from inorganic Se (Sunde, 1997).

Dietary selenomethionine can be readily incorporated into protein ([Se]Met) as selenomethionine because it is esterified to methioninyl-tRNA only slightly less efficiently as Met (Hoffman*et al.,* 1970; McConnell and Hoffman, 1972). Selenomethionine can be metabolized to Se-adenosylmethionine (SeAM), and then to Se-adenosylhomocysteine (SeAH; Markham *et al.,* 1980). The SeAH is readily converted to selenocysteine via cystathionineɓ-synthase and cystathionineↄ-lyase. Selenocysteine then can be incorporated into proteins or degraded, releasing selenite, or it can be degraded by selenocysteinelyase, releasing elemental Se (Se-), which can be reduced to selenide (Esaki *et al.,* 1982). Another potential fate for selenomethionine is to be transaminated to methaneselenol (Steele and Benevenga, 1979), and then methaneselenol can be transformed to selenide via S methyltransferase (Sunde, 1997). At this point, selenide would be metabolized as discussed above.

Sturniolo *et al.* (2000) reported different absorption pathways (active transport or passive diffusion) and bioavailability derive from the different forms of dietary Se supplementation (organic or inorganic). Absorption of Se in the gastrointestinal tract is efficient, with uptake of around 80% (Abdel-Azeem, 2010). Selenomethionine appears to be

actively absorbed, sharing a transport mechanism with the amino acid methionine. Selenocysteine may also share a common active transport mechanism with basic amino acids. Selenate is absorbed by a sodium-mediated carrier transport mechanism shared with sulphur, while selenite uses passive diffusion. A number of dietary factors, in addition to the chemical form, can affect absorption from food (Reilly, 2004). Therefore, the rate-limiting step determining the overall availability of dietary Se is not likely to be its absorption but rather its conversion within tissues to its metabolically active forms (WHO and FAO, 2004). Inorganic Se sources undergo reduction to form selenide which leads to the formation of Se-Cysteine (i.e., the hydroxyl group of a serine molecule linked to a specific tRNA [UGA codon] is replaced with a selenol moiety to form Se-Cyst-tRNA that is inserted into selenoproteins). Thus various Se sources (both inorganic and organic) must first be converted to inorganic selenide before the synthesis of Se-Cysteine which contribute to the bioactive components of Selenoproteins. Following absorption of Se-Methionine from the intestinal tract, Se-methionine can be found in blood proteins and in the plasma methionine pool as it is transported to body tissues. For example the mammary gland extracts large quantities of methionine to synthesize milk proteins. This would account for the large amounts of Se found in milk which may benefit the neonate or serve as a Se source for human consumption (Abdel-Azeem, 2010).

2.1.4. Dietary Sources of Selenium

The dietary sources of Se for pigs can be divided into two groups: natural feedstuffs, such as corn or soybean meal, and supplemental sources, such as sodium selenite. The Se levels in plant-based feedstuffs vary greatly depending on plant species and soil status (Levander, 1986). Plants then can be divided into two groups based on their ability to accumulate Se. Examples of Se-accumulator plants are species of *Astragalus and Neptunia*, which can have Se levels well over 1,000ppm (Levander, 1986). However, these Se-

accumulator plants are not commonly used in animal diets. Selenium non-accumulator plants, such as corn, wheat, or oats, are more typical of the feedstuffs used for animal diets. Pastures and forages grown in areas where animals do not seem to have any Se-deficiency-related conditions usually have levels of 0.10 ppm Se or greater (Levander, 1986).

However, areas where animals do express signs of these conditions often have Se levels of 0.05 ppm or lower (Levander, 1986). Also, there is considerable difference in the Se level of grains, with the extremes being wheat containing 0.32 ppm of Se or oats containing 0.20 ppm of Se (Levander, 1986). Beilstein and Whanger (1986) indicated that the major form of Se in non-accumulator plants was selenomethionine (SM). Peterson and Butler (1962) and Olson *et al.* (1970) indicated that Se existed in several forms, including SM, selenocysteine, or Se-methylselenomethionine, in typical forages and seeds consumed by animals. Natural feedstuffs made from animal products also contain Se, and the Se found in these sources is predominantly selenocysteine (Hawkes *et al.,* 1985). Both Scott and Thompson (1971) and Gabrielsen and Opstvedt (1980) reported that various fish meals contained relatively high levels of Se, ranging from 1.3 to 6.2 ppm.

The supplemental sources of Se vary in their ability to meet the requirement of the animal due to chemical form, and how that form is metabolized once it is absorbed. Schwarz and Foltz (1957) divided these sources into three categories according to their potency against liver necrosis in rats. The first category contains elemental Se, which is poorly absorbed by the animal. The inorganic salts, such as selenites, selenates, and the Se analogs of methionine and cysteine, make up the second category. Originally, Schwarz and Foltz (1957) reported that there were no differences in the ability of these sources to protect against liver necrosis. However, as indicated above, Noguchi *et al.* (1973) and Cantor *et al.* (1975) indicated differences in their ability to alleviate Se-deficiency conditions within this group of compounds. The third category contains organic Se compounds that are more active than

those in the second group. These sources have not been well researched because of their chemical instability and low yields.

2.2 Selenium and Reproduction in Pigs

2.2.1. Selenium and pregnancy in sows (gestation, litter size, sow body weight gain at gestation)

Fundamental processes of animal reproduction are compromised by Se deficiency: infertility due to fragility and dysmotility of sperm, embryonic loss before implantation, stillbirths, poor intrauterine growth, prematurity, placenta retention, and poor postnatal growth (Scholl and Reilly, 2000)

Selenium deficiency adversely affects reproduction in both sexes and all species including humans (MacPherson, 1994). The problem is most extensively described in cattle and sheep and while there have been reports of Se deficiency as a direct cause of abortion, the related problems of increased disease susceptibility and retained placenta make it difficult to view infertility in the dam in terms of a single factor (Maas, 1998). While the role of Se status in female fertility is more recognized than understood, recent work has provided much clearer insight into the well known association between Se and male fertility through identification of the membrane-bound phospholipid hydroperoxide glutathione peroxidase (GSH-Px) in the testes (Jacques, 2007).

Indeed, Se is an essential nutrient in that Se deficiency is associated with increased incidences of retained foetal membranes, clinical mastitis, calf mortality and increased milk somatic cell counts (Abdel-Azeem, 2010). Selenium supplementation in Se deficient diets reduced the incidence of these clinical problems. Retained placentas are the most common reproductive problem associated with Se deficiency. But early embryonic deaths, increased metritis (an infection of the uterus), poor fertility, and the birth of dead or weak calves also are associated with low levels of Se (Amaral-Phillips and Heersche, 1997).

The improvement in conception rate seen in some studies but not others (Hostetler *et al.,* 2003) may be due to a down-stream effect resulting from the reduction of retained placenta, reduced incidence of metritis and fewer days to insemination and conception following Se supplementation. The discrepancy in litter size observed in these studies could arise from many factors including maternal age, timing and duration of supplementation, level of Se in the basal diet, the level of Selenium supplementation or the source of Se (Hostetler *et al.,* 2003).

Mahan and Kim (1996) reported that Se source had no effect on gilt reproductive performance so, gilt weights and weight changes that occurred during gestation and lactation were not influenced by dietary Se source. Selenium source had no effect on lactation feed intake. The number of pigs born (total, live and stillborn) and individual pig birth weights were not affected by dietary Se source. Litter size, the number of piglet mortality from 0 to 7days postpartum, and litter and pig weights at both 7 and 21 days were not influenced by dietary Se source. Also, Svoboda *et al.* (2008 and 2009) reported that the number of piglets born (total, live and stillborn) was not affected by the form of Se supplement (organic vs. inorganic).

2.2.2. Selenium and lactation in sows

Ambient temperature can affect the milk output of the lactating sow. In hot environment, sow feed intake and milk production decrease to prevent a rise in body temperature (Quiniou and Noblet, 1999; Renaudeau and Noblet, 2001). Exposing lactating sows to heat stress which subsequently lead to oxidative stress by the accumulation of reactive oxygen species (ROS) reduces their voluntary feed intake (VFI), as well as nutrient availability for milk synthesis, causing a decline in milk yield and a decrease in litter growth. This decrease in milk production may also be caused by a change in endocrine function that reduces the nutrient supply to the mammary gland during heat stress. Thyroid hormones, such

as T4 and T3, are key metabolic hormones of the body and associated with protein synthesis and energy production, with T3 being the most functional active form (Smith *et al.,* 2002 Zhan *et al.,* 2007a). Thyroid hormones and cortisol regulate nutrient partitioning toward milk production. During heat and oxidative stress there is a decrease in the circulating concentrations of these hormones which limits body reserve mobilization, thereby leading to decreased milk production (Messias de Bragança *et al.,* 1998).

Selenium is an important component of selenoprotein enzyme (i.e. GSH-Px) in mammalian tissues and also an integral part of the enzyme type 1 iodothyrionine deiodinase, which catalyses the deiodination of the iodothyronines notably the deiodination of T4 to triiodothyronine (T3), the most active of the thyroid hormones (Arthur *et al.,* 1993). It was widely accepted that proper Se intake improved the antioxidant status of the body thereby reducing the harmful effect of heat and oxidative stress which in turn improves the availability for milk production causing an increase in milk yield.

2.2.3. Effect of selenium on Thyroid hormone secretion

Selenium is an integral part of the enzyme type 1 iodothyrionine deiodinase, which catalyses the deiodination of the iodothyronines notably the deiodination of T4 to triiodothyronine (T3), the most active of the thyroid hormones (Arthur, 1993). This deiodination is also catalysed by type II and type III deiodinases, which are not selenoproteins. While all deiodinases catalyse the conversion of T4 to T3, there are differences in the tissue distribution of these enzymes (Carolyn, 2013).

Iodothyronine Deiodinases (ID) is the second largest group of selenoproteins. The three deiodinases (Type I, II, and III ID) control the local availability and concentration of the active thyroid hormone, 3, 3, 5-triiodothyronine (T3). These enzymes catalyze the conversion of thyroxin (T 4) to T3 (Type I and II ID) or the deiodination of T4 and T3 to non-active

metabolites (Type III ID). These three isoenzymes are encoded by different genes and have tissue and development-specific patterns of expression and regulation (Kohrle *et al.*, 2000). In general iodothyronine deiodinase (ID) is ranked higher in priority for available Selenium supply than is cytosolic GSH-Px and was similar in ranking to that for GSH-Px-PH and selenoprotein (Kohrle, *et al.*, 2005). Because thyroid hormone controls growth, development, differentiation and many metabolic reactions, Selenium is believed to be involved in regulation of those functions as well.

In Se-deficient animals, type I ID synthesis is markedly impaired and this impairment is reversed when selenium is restored to the diet. Under these same conditions, the ratio of T3 to T4 is altered. There is more T4 and less T3 in selenium deficient animals and the ratio of the two is reversed when selenium is restored. Reduced ID I activity in the pituitary is associated with lower levels of growth hormone in Se-deficient animals (MacPherson, 1994). When T4 level rises (as in selenium deficiency), this rise feeds back to the pituitary, which in turn alters (reduces) thyroid-stimulating hormone (TSH) release. The conversion of T4 to T3 in the pituitary is catalysed by the type II deiodinase, yet TSH release falls. T4 levels are high because the type I deiodinase is less active. Whereas deficient animals might have a T3/T4 ratio of 0.01, the selenium-sufficient animal has a ratio of 0.02 a doubling of the conversion of T4 to T3. The effect of selenium supplementation in the synthesis and activity of the type I deiodinase probably explains the poor growth of deficient animals. Bermano *et al.* (1996) have reported significant linear growth in deficient rats given a single selenium supplement and this growth was directly related to the supplement induced increase in type I deiodinase

Thyroid hormone activity is a key factor in animal tolerance to cold stress. A well-known thyroid hormone function is the heat-producing increase in oxygen consumption of tissues in response to cold temperatures

2.2.4. Selenium and growth of piglets

The growth of piglets depends largely on the milk producing ability of the sow and its availability to the piglet. It is well known that selenium plays a vital role in increasing milk yield of sows. Apart from milk yield and its availability, the antioxidant status of the piglet is also important. At birth, the antioxidant status of piglet is low, and increases as the pig age advances (Mahan, 2000). Also obvious is that an animal body with low antioxidant status are easily prone to disease, and thus interacts negatively with the growth performance (Zhan *et al.,* 2006). Maternal selenium intake could provide one of the effective feeding methods to improve the antioxidant status of piglets, and thus benefit the health of piglets. Selenium supplementation is effective for facilitating the conversion T4 to T3 through the action of selenoenzyme (i.e. enzyme type udeiodinase) (Bianco, *et al.,* 2002). This greatly increased serum T3, while serum T4 was greatly decreased. More T4 deiodinated to the active T3 indicated a promoted protein synthesis and energy production in piglet of selenium-treated groups. Selenium supplementation According to Xu *et al* (2002), digestion of nutrients is affected by the digestive enzyme activity in the small intestine. The activities of pancreatic digestive enzymes are closely associated with digestion and absorption of nutrients, and thus affect the growth performance. Maternal selenium intake greatly increases protease, amylase and lipase activities in the pancreatic tissue of its offspring and enhance digestion of nutrients in piglets, which increases the growth performance of piglets from birth to 28th weaning day.

2.3 Selenium and Oxidative Enzyme Secretion or Metabolism in Pigs

2.3.1. Glutathione Peroxidases.

Glutathione peroxidase (GSH-Px) is a mammalian selenoenzyme which functions as a catalytic antioxidant and protects various organisms from oxidative stress and cellular membrane damage (Ange Mouithys *et al.,* 2004). GSH-Px catalyses the reduction of harmful

peroxides using glutathione (GSH) as the reducing substrate according to the following equation:

ROOH + 2GSH Ÿ ROH + GSSG + H2O.

Ursini *et al* (1995) described four structurally and genetically different forms of Se-containing GSH-Px that exist in different tissues or parts of the cell. The most abundant selenoprotein in mammalian tissues is the cytosolic GSH-Px (cGSH-Px). This classical form of GSH-Px efficiently metabolizes hydrogen peroxide as well as unesterified fatty acid hydroperoxides in conjunction with GSH, the pentose phosphate cycle, and glutathione reductase (GR) (Wolffram, 1999). cGSH-Px is a tetrameric protein with four identical subunits, each containing one Se atom (Sunde, 1997). The second cytosolic GSHPx referred to as GSH-Px-GI which is found in the gastrointestinal tract cells. The enzymatic properties of this form are practically the same as those of cytosolic GSH-Px. The physical properties of the two are also similar, with the activity of both depending on Se supply (Chu *et al.,* 1993). The third form, which is extracellular, is present in the plasma and denoted as pGSH-Px (Daniels, 1996).

Phospholipid hydroperoxide GSH-Px (GSH-Px-PH), a membrane-bound form of GSH-Px, plays an important role in the interaction of vitamin E and Se. It has been postulated that GSH-Px-PH along with vitamin E acts as a chain-breaking antioxidant to protect phospholipid membranes. GSH-Px-PH may also be involved in regulation of leukotriene biosynthesis. This enzyme is preferentially expressed in endocrine and reproductive tissues and has been shown to play a major role in male reproduction (Kohrle *et al*., 2000). Ursini *et al.* (1995) found that in the testis, the GSH-Px-PH acts as a powerful antioxidant in the developing spermatids and spermatozoa. GSH-Px-PH is distinguished from classical GSH-Px as it is active in monomeric form and has a different amino acid composition (Sunde, 1997).

2.3.2. Superoxide dismutase

Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defence in nearly all cells exposed to oxygen. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and the Ni type, which binds nickel. (McCord and Fridovich, 1969)

- Copper and zinc SOD ï most commonly used by eukaryotes. The cytosols of virtually all eukaryotic cells contain an SOD enzyme with copper and zinc (Cu-Zn-SOD). For example, Cu-Zn-SOD available commercially is normally purified from the bovine erythrocytes. The bovine Cu-Zn protein was the first SOD structure to be solved (Richardson *et al.,* 1975). It is an 8-stranded "Greek key" beta-barrel, with the active site held between the barrel and two surface loops. The two subunits are tightly joined back-to-back, primarily by hydrophobic and some electrostatic interactions. The ligands of the copper and zinc are six histidine and one aspartate side-chains; one histidine is shared between the two metals (Tainer *et al.,* 1983).
- Iron and manganese SOD ï used by prokaryotes and protists, and in mitochondria. *E. coli* and many other bacteria also contain a form of the enzyme with iron (Fe-SOD); some bacteria contain Fe-SOD, others Mn-SOD, and some contain both. Fe-SOD can be found in the plastids of plants. The 3D structures of the homologous Mn and Fe superoxide dismutases have the same arrangement of alpha-helices, and their active sites contain the same type and arrangement of amino acid side-chains. Chicken liver and nearly all other mitochondria, and many bacteria (such as *E. coli*), contain a form of SOD with manganese (Mn-SOD): for example, the Mn-SOD found in human mitochondria. (Borgstahl *et al.,* 1992)

2.3.3. Catalase metabolism

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. (Chelikani *et al.,* 2004) It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second. (Goodsell, 2004)

Hydrogen peroxide is a harmful by-product of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules. (Gaetani *et al.,* 1996)

The reaction of catalase in the decomposition of living tissue:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Catalase can also catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction:

$H_2O_2 + H_2R \rightarrow 2H_2O + R$

The exact mechanism of this reaction is not known.

The presence of catalase in a microbial or tissue sample can be tested by adding a volume of hydrogen peroxide and observing the reaction. The formation of bubbles, oxygen, indicates a positive result. This easy assay, which can be seen with the naked eye, without the aid of instruments, is possible because catalase has a very high specific activity, which produces a detectable response.

2.3.4. Malondialdehyde (MDA)

Malondialdehyde, MDA, is the organic compound with the formula $CH_2(CHO)_2$. This reactive species occurs naturally and is a marker for oxidative stress Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (Farmer and Davoine, 2007). Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, including MDA. It can also be formed during arachidonic acid metabolism for the synthesis of prostaglandins (Marnette, 1999) catalyzed by thromboxane synthase and in liver cells (Plastaras *et al.,*2000) by breakdown of prostaglandin endoperoxide. MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA (Sevilla *et al.,* 1997).

2.3.5. Glutathione Reductase.

Glutathione Reductase (GR) is a ubiquitous enzyme that catalyses the reduction of oxidized glutathione (GSSG) to GSH in the presence of reduced NADPH. GR is essential for the GSH redox cycle that maintains adequate levels of reduced cellular GSH, the predominant form in the tissues, which serves as an antioxidant reacting with free radicals and organic peroxides (Rall and Lehninger, 1952). It is generally accepted that GR is largely responsible for the maintenance of intracellular glutathione in the reduced form. However, the enzyme also functions intracellulary in the reduction of mixed disulphides of GSH and protein.

GR is an important enzyme associated with the hexose monophosphate pathway in erythrocytes (Harris and Kellermeyer, 1970). Research has shown GR to be a flavin enzyme with flavin dinucleotide (FAD) serving as an apparent prosthetic group (Scott *et al.,* 1963; Buzard and Kopko, 1963; Staal *et al.,* 1969; Staal and Veeger, 1969).

The Se-dependent GSH-Px system, which is vital for the protection against tissue peroxidation, requires the following nutrients: Se as a component of the GSH-Px, the sulphur amino acids in the form of cysteine in GSH, riboflavin in FAD for GR activity and niacin as a component of reduced NADPH.

Both GSH-Px and GR are involved in the cycling of glutathione. GSH-Px oxidizes glutathione while reducing toxic endogenous and exogenous peroxides while GR restores the reduced glutathione status. GR activity may be associated with the requirement for the accumulation of oxidized GSH, which is the product of the reaction catalyzed by GSH-Px. (Pablos *et al.,* 1998).

Studies with humans (Beutler, 1969; Bamji, 1969; Tillotson and Baker, 1972) and rats (Glatzle *et al.,* 1968; Srivastava and Beutler, 1970; Buzard and Srivastava, 1970) demonstrated that measurement of erythrocyte GR (EGR) activity would be a useful and sensitive procedure for evaluating the riboflavin status of individual subjects or population groups. The EGR activity is altered in vivo by dietary riboflavin and in vitro by FAD. The degree of in vitro stimulation or EGR activity is dependent on the FAD saturation of the apoprotein, which in turn is dependent on the availability of riboflavin. The action of GR is also critically dependent on an adequate supply of NADPH (Godin and Garnett, 1992).

Rutz *et al.* (2003) reported that whole blood GR was reduced in broilers receiving low riboflavin diets. These authors later reported a significant interaction of Se and riboflavin on erythrocyte GR activity coefficient (Rutz *et al.,* 2003), both Se and riboflavin decreased the coefficient. Brady *et al.* (1979) reported that erythrocyte GR activity, but not hepatic and muscle GR activity, was increased by riboflavin supplementation in piglets. Parsons *et al.* (1985) reported that percentage active GR declined rapidly when pigs were placed on a riboflavin-unsupplemented diet and was lower than that of riboflavin-supplemented pig diet.

There are large differences in the activity of both plasma and erythrocyte activity among species reported in the literature. Unlike erythrocyte, plasma GR is not significantly stimulated by the addition of FAD. In species with high plasma GR activity the determination of whole blood FAD activity coefficients may not be a good index of riboflavin status (Board and Peter, 1976).

2.3.6. Selenium as an Antioxidant

Most animals, plants, and microorganisms rely on oxygen for the efficient production of energy. However, the high oxygen concentration in the atmosphere is potentially toxic for living organisms because free radicals derived from oxygen can damage many types of biological molecules. The presence of natural antioxidants in living organisms enables the survival in an oxygen-rich environment (Halliwell, 1994).

The formation of free radicals is a pathobiochemical mechanism involved in the initiation or progression of various diseases (Hogg, 1998). In livestock production, free radical generation and lipid peroxidation are responsible for the development of various diseases, decreases in animal productivity, and product quality (Hurley and Doane, 1989; Weiss, 1998; McDowell, 2000).

A variety of different stress conditions are associated with the over-production of free radicals and thus cause a disturbance in the balance of pro oxidant-antioxidant leading to the potential for tissue damage (Jaeschke, 1995). Stress conditions are usually divided into three main categories: nutritional stress, environmental conditions, and internal stress. All of these conditions stimulate free radical generation by decreasing the coupling of oxidation and phosphorylation in the mitochondria that results in an increased electron leakage and over production of superoxide radical (Dalton *et al.,* 1999).

Once free radical production exceeds the antioxidant systems capacity to neutralize it, lipid peroxidation causes damage to unsaturated lipids in cell membranes, amino acids in proteins, and nucleotides in DNA. Thus resulting in membrane and cell integrity disruption. This inevitably results in decreased productive and reproductive performance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and duration of the Study

The study was carried out at the Piggery Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka lies in the Derived Savannah region, and is located on longitude 6^{o} 25˜N and latitude 7^{o} 24˜E (Offomata, 1975) and at an altitude of 430m above sea level (Breinholt *et al*., 1981). The climate of the study area is a typical humid tropical type, with a relative humidity range of 56.01 to 103.83%. Average diurnal minimum temperature ranges from 22 to 24.7 ^{o}C while the average maximum temperature ranges from 33 to 37 ^{o}C (Okonkwo and Akubuo, 2007). Annual rainfall ranges from 1567.05 to 1846.98 mm (Meteorological centre, Crop Science Department University of Nigeria Nsukka, 2009 unpublished). The study lasted for five months.

3.2 Experimental Materials

Spring Valley Selenium Dietary Supplement Tablets, 200micrograms, 100 count distributed by Walmart Stores Inc. Bentonville, AR, United States of America was used for the study.

3.3 Experimental Animals and Management

Twelve (12) crossbred sows (Landrace × Large White) aged 1 to 2 years with an average body weight of 56.1 kg were used for this work. The sows were sourced from a commercial farm in Nsukka. The experimental animals were housed in half walled pens in an open sided, naturally ventilated building with concrete floor and asbestos roofing sheets. A week before the arrival of the animals, the pens were cleaned and subsequently disinfected to reduce the risk of disease infection. On arrival the animals were dewormed with Ivomec and

provided with a week period of equilibration to acclimatize to the environment, feed and water. Cleaning of experimental pens was performed every morning. The piglets were weaned five (5) weeks after birth.

3.4 Experimental Design

The experimental design was a Completely Randomised Design (CRD). The 12 sows were randomly assigned to four (4) treatment groups (T_0, T_1, T_2, and T_3) with 3 sows each per treatment. Each sow was housed individually in pen and served as a replicate. The T_0 served as the control group and received diet devoid of Se while T_1, T_2 T_3 were supplemented with 0.3 mgSe/kg feed in the second trimester of gestation (30 ï 75 days post-coitum), third trimester of gestation (75 days post-coitum to term) and lactation period (0 ï 30 days after farrowing). Each animal received 2.20 kg of feed as ration (Table 1) at 08:00hrs while cool clean drinking water was provided *ad libitum*.

Below is the experimental model:

$$\mathbf{Y_{ij} = U + A_i + E_{ij}}$$

Where: $\mathbf{Y_{ij}}$ = individual observation

U = population mean

$\mathbf{A_i}$ = effect of selenium supplementation

$\mathbf{E_{ij}}$ = experimental or random error

3.5 Experimental Diets

The composition of experimental diets are presented in Tables 2 and 3

Table 2: Percentage Composition of experimental diet

Ingredient	**Quantity**
Wheat offal	25.00
PKC	28.00
GNC	15.00
Maize	30.00
Limestone	2.00
Bone meal	2.20
Lysine	0.25
Methionine	0.25
Salt	0.20
Enzyme + Toxin binder	0.10
Total	**100**
Calculated	
Crude protein (%)	18.33
Energy (Kcal/Kg ME)	2627.03

Table 3: Proximate Composition of experimental diet

Components	Percentage
Dry matter	90.90
Crude protein	19.51
Crude fibre	8.51
Ash	10.00
Ether extract	5.50
Moisture	9.10
NFE	47.38
Energy (Kcal/Kg)	2814.38

3.6 Data Collection

3.6.1 The parameters measured

i. Sow body weight at mating

ii. Sow body weight during gestation

iii. Sow body weight at parturition

iv. Sow body weight during lactation

v. Litter size at birth

vi. Piglet birth weight

vii. Litter size at weaning

viii. Piglet weaning weight

ix. Mortality

x. Weaning percentage

Haematological indices

i. Haemoglobin (HB)

ii. Packed Cell Volume (PCV)

iii. White Blood Cell (WBC)

iv. Red Blood Cell (RBC)

Blood biochemistry

i. Glutathione peroxidise

ii. Reduced glutathione

iii. Selenium in blood

iv. Malondialdehyde

v. Catalase

vi. Superoxide dismutase (SOD)

3.7 Blood collection and Analysis

Blood was collected on the 30th day from the sows after receiving the treatment and subsequently after every 30 days at each stage of gestation. Five (5) millilitre of blood was drawn from the ear vein using a sterile disposable 5ml syringe. The blood samples were divided into two, 2.5 ml each. The first sample (2.5 ml) was used for haematological determination and was poured into an (ethylene diamine tetra-acetic acid) EDTA bottle. The remaining 2.5 ml was used for serum biochemical indices and was collected into anticoagulant free test tubes and later centrifuged at 3000 revolutions per minute (rpm) for 10 minutes at room temperature. The serum was separated into different test tubes leaving the thick blood residue and serum was then subjected to analysis.

3.8. Methods of Analysis for Haematogical Parameters

(1) **Haemoglobin (Hb%)**

0.02ml of well mixed whole blood was diluted into four (4) mls of drabkins solution. This is measured spectrophotometrically at 546 nanometer wavelength. After measuring the value of Hb was read from the calibration curve (calorimetrically) according to Van Kampen and Zillestra (1983).

(2) **Pack Cell Volume (PCV)**

Method:

The PCV was determined by the microheamatocrit method (Coles, 1980). A micro capillary tube was nearly filled with the blood sample and sealed at one end. It was centrifuged at 10,000 revolutions per minute for 5 minutes using a microheamatocrit centrifuge. After centrifugation, the PCV was read using a microheamatocrit reader.

(3) **WBC (mm^3) total white blood cell count**

Method:

0.02ml of blood was diluted with 0.38ml of diluting fluid. The total blood count is counted using improved neubaur counting chamber.

(4) **RBCs – Red blood cells count:**

Method:

All counting methods are based on the dilution of capillary blood or well mixed, correctly anti-coagulated venous blood with counting fluids in a special counting pipette. The individual cells are counted in a counting chamber (hemocytometer).

3.8.1 Oxidative Enzyme Parameters Measured were

Assay of catalase

Method:

Catalase was assayed according to the method of Aebi (1983). In a test tube, I Pipetted 2.5 ml of phosphate buffer (pH 7.0), 0.5ml of sample and 2 ml of hydrogen peroxide solution. To 1 ml of the reaction, 2 ml of dichromate acetic acid reagent was added. The decrease in absorbance was measured at 240nm at a minute interval up to 4 minutes. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as µmoles of hydrogen peroxide decomposed/minute/mg of protein.

Superoxide Dismutase (SOD)

Method:

Superoxide dismutase was assayed according to the method of Xin *et al.,* (1991) 0.1ml of sample was added to the test tube containing 0.9 ml of distilled water. 0.1 ml of this mix was then mixed with 0.9 ml of carbonate buffer (pH 10.2) and 75µl of Xanthine oxidase. The absorbance was determined at 480nm in a Shimadzu UV spectrophotometer at 30

seconds interval for 3 minutes. One unit of superoxide dismutase activity is related to the amount of protein required for 50% inhibition of the detector reaction

Glutathione Peroxidase

Method:

Glutathione Peroxidase activity was measured by following the rate of NADPH oxidation at 340 nm and by the coupled reaction with glutathione reductase (Bell *et al.,* 1985).

Total Reduced glutathione

Total reduced glutathione was determined by the method of Sedlak and Lindsay (1968) modified according to the method of Moron *et al.,* (1979). 0.1ml of the test sample was precipitated with 5% TCA. The precipitate was removed by centrifugation. To 2.0ml of the supernatant, 2.0ml of 0.6mg of DTNB was added in 0.2M phosphate buffer (pH 6.5). The absorbance was read at 412nm against a blank containing TCA instead of sample. A series of standards treated in a similar manner were also run to determine the glutathione content. The amount of glutathione was expressed as mg/dl for plasma and mg/100g of tissues.

Selenium

Method:

0.1ml of the sample was pipetted and added to 0.9g of distilled H_20. 5ml of concentrated hydrogen chloride was also added and the mixture shaken. 2ml of 2, 4, DNPH ï NEDA reagent was also added and the mixture was allowed to stand for 10 minutes with occasional shaking. The mixture was diluted to 10ml with distilled water and the absorbance Measured at 520nm

Assay of Lipid Peroxidation (LPO) – Malondialdehyde (MDA)

Malondialdehyde contents were estimated according the method of Wallin *et al.,* (1993). 0.1 ml of the sample in a test tube was added 0.9 ml of distilled water, 0.5 ml of 25%

TCA and 0.5 ml of 1% TBA in 0.3% NaOH and mixed thoroughly. The solution was heated for 40 minutes at 95 ^{0}C in a boiling water bath. After cooling, 0.1ml of 20% SDS (Sodium dodecylsulphate) was added. The absorbance was determined at 532nm and 600nm against a blank that contains all the reagents except the sample.

3.9 Statistical Analysis

The data collected was subjected to analysis of variance (ANOVA) using the SPSS computer package in accordance with a completely randomized design (CRD). Significant differences found among the treatment means was separated using Duncanôs New Multiple Range Test (Duncan, 1955) and accepted at 5% or 1% level of probability.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of dietary selenium supplementation on sow reproductive performance and preweaning growth performance of piglets

The results of the effect of dietary selenium supplementation on sow reproductive performance and preweaning growth performance of piglets are shown in Tables 4 and 5, respectively.

Table 4: Effect of dietary selenium supplementation on sow reproductive performance and preweaning growth performance of piglets (Mean ± SE)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
Sow body weight at mating(kg)	57.50 ± 5.92	58.70 ± 0.02	59.00 ± 2.64	60.67 ± 1.85	0.16^{NS}
Sow body weight during gestation(kg)	84.17 ± 6.46	97.67 ± 6.74	87.67 ± 8.09	83.00 ± 3.61	0.41^{NS}
Sow body weight at farrowing(kg)	83.00 ± 6.42	94.33 ± 5.78	84.33 ± 8.29	79.33 ± 3.75	0.12^{NS}
Sow gestation weight gain (kg)	26.50 ± 6.78	39.00 ± 6.94	28.67 ± 5.45	22.33 ± 1.76	0.56^{NS}
Sow body weight loss during lactation(kg)	15.33 ± 5.69	17.33 ± 1.76	11.67 ± 2.33	9.67 ± 5.36	0.10^{NS}
Litter size at birth	5.67 ± 0.33	6.00 ± 92.47	7.66 ± 1.20	8.33 ± 8.33	0.49^{NS}
Litter size at weaning	3.33 ± 0.33^{c}	5.00 ± 79.73^{b}	7.00 ± 1.16^{a}	7.86 ± 0.67^{a}	0.03 *
Piglet mortality (%)	2.33 ± 0.33^{a}	1.00 ± 0.49^{b}	0.66 ± 0.33^{b}	0.47 ± 3.05^{b}	0.04 *
piglet birth weight (kg/piglet)	1.00± 0.96	0.99±0.16	1.07± 0.02	1.16 ± 0.07	0.42^{NS}
weight gain week 1(kg)	0.72± 0.07	1.26 ± 0.53	1.09 ± 0.21	0.72 ± 0.15	0.69^{NS}
weight gain week 2(kg)	1.19 ± 0.10	0.90 ± 0.15	1.12 ± 0.05	1.21 ± 0.10	0.90^{NS}
weight gain week 3(kg)	0.90 ± 0.47^{b}	0.86 ± 0.11^{b}	1.22 ± 0.16^{ab}	1.37 ± 0.19^{a}	0.001**
weight gain week 4(kg)	1.25 ± 0.16^{c}	1.78 ± 0.52^{a}	1.25 ± 0.09^{c}	1.38 ± 0.15^{b}	0.01**
Preweaning wt gain of piglets (kg)	4.07 ± 2.00^{c}	4.80 ± 0.98^{a}	$4.69 \pm .0.49^{b}$	4.77 ± 0.64^{ab}	0.03*
Weaning weight (kg/piglet)	5.07 ± 5.92^{c}	5.79 ± 0.02^{b}	5.76 ± 2.64^{b}	5.93 ± 1.85^{a}	0.01**

[a,b,c] ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05; ** P<0.01), NS- Not significant.

Sow body weight during gestation (kg):

The effect of selenium supplementation was not significant (P>0.05) on sow body weight during gestation Average body weight of sows in T_0 (84.17 ± 6.46kg), T_1 (97.67 ± 6.74kg), T_2 (87.67 ± 8.09kg) and T_3 (83.00 ± 3.61kg) during gestation were similar (P>0.05) among treatments.

Sow body weight at farrowing (kg):

Selenium supplementation had no significant (P>0.05) effect in body weight at farrowing among treatments. Average body weight of sows at farrowing were T_0 (83.00 ± 6.42kg), T_1 (94.33 ± 5.78kg), T_2 (84.33 ± 8.29kg) and T_3 (79.33 ± 3.75kg). Results showed no significant effect (P>0.05) of dietary selenium supplementation among treatments.

Sow gestation weight gain (kg):

Selenium supplementation had no significant (P>0.05) effect in sow gestation weight gain among treatments. Gestation weight gains of sows were T_0 (26.50 ± 6.78kg), T_1 (39.00 ± 6.94kg), T_2 (28.67 ± 5.45kg) and T_3 (22.33 ± 1.76 kg). Results showed no significant effect (P>0.05) of dietary selenium supplementation among treatments.

Sow body weight loss during lactation (kg):

Selenium supplementation had no significant (P >0.05) effect in sow body weight loss during lactation. Lactation weight loss of sows were T_0 (15.33 ± 5.69kg), T_1 (17.33 ± 1.76kg), T_2 (11.67 ± 2.33kg) and T_3 (9.67 ± 5.36kg). Results showed no significant effect (P>0.05) of dietary selenium supplementation among treatments.

Litter size at birth:

Dietary Selenium supplementation had no significant (P>0.05) effect on litter size at birth. Litter size of sows on T_0, T_1, T_2 and T_3 were 5.67 ± 0.33, 6.00± 92.47, 7.66 ± 1.20 and 8.33 ± 8.33, respectively.

Litter size at weaning:

A significant effect ($P<0.05$) of selenium supplementation was shown on litter size at weaning. Litter size at weaning was higher for sows on T_2 (7.00 ± 1.16) and T_3 (7.86 ± 0.67) and it differed significantly ($P<0.05$) from those of sows on T_0 (3.33 ± 0.33) and T_1 (4.33 ± 0.67).

Piglet mortality (%):

Dietary Selenium supplementation had significant effect ($P<0.05$) on Piglet mortality. Piglet mortality was higher for sows on T_0 (2.33 ± 0.33) and it differed significantly ($P<0.05$) from those of sows on T_1 (1.00 ± 0.49) T_2 (0.66 ± 0.33) and T_3 (0.47 ± 3.05) which had similar and comparable ($P>0.05$) values.

Piglet birth weight (kg):

No significant ($P>0.05$) effect of selenium supplementation on birth weight of piglets was shown among treatments. Average body weight of piglets at birth were T_0 (1.00 ± 0.96kg), T_1 (0.99 ± 0.16 kg), T_2 (1.07± 0.02kg) and T_3 (1.16 ± 0.07kg).

Piglet weight week 1 and week 2 (kg):

In week 1 and 2 weekly weight gain of piglets from the different treatments was not significantly different ($P>0.05$) as shown in Table 4. Average weight gain of piglets at week 1 and 2 were T_0 (0.72± 0.07kg), T_1 (1.26 ± 0.53kg), T_2 (1.09 ± 0.21kg) T_3 (0.72 ± 0.15kg) and T_0 (1.19 ± 0.10kg), T_1 (0.90 ± 0.15 kg), T_2 (1.12 ± 0.05kg) and T_3 (1.21 ± 0.10kg) respectively.

Piglet weight week 3 (kg):

Selenium supplementation had highly significant ($P<0.01$) effect on week 3 weight gain of piglets. Piglets on T_3 (1.37±0.19kg) had the highest body weight gain at the third week and it differed significantly ($P<0.05$) from those on T_0 (0.90 ± 0.47kg) and T_1 (0.86±0.11kg). However, weight gain for piglets on T_2 (1.22 ± 0.16kg) and T_3 was similar ($P>0.05$) whereas those on T_0 T_1 and T_3 were also comparable ($P>0.05$).

Piglet weight week 4 (kg):

Selenium supplementation on week 4 weight gain of piglets was highly significant ($P<0.01$). Piglets on T_1 Sows (1.78±0.52kg) had the highest body weight gain which differed significantly ($P<0.05$) from those on T_3 (1.38±0.15kg), T_2 (1.25±0.09kg) and T_0 (1.247±0.16kg). However, Piglets weight gain of T_3 differed ($P<0.05$) from those in T_0 and T_2 which were comparable statistically ($P>0.05$).

Preweaning weight gain of piglets (kg):

The effect of Selenium supplementation on Preweaning weight gain of piglets was significant ($P<0.05$). Piglets on T_1 (4.80± 0.98kg) had the highest preweaning weight gain and it differed significantly ($P<0.05$) from those on T_2 (4.69± .049kg) and T_0 (4.07± 2.00kg) but similar to those of T_3 (4.77± 0.64 kg). However, preweaning weight gain of T_3 and T_2 are comparable but differed ($P<0.05$) from those in T_0 which had the lowest preweaning weight gain.

Weaning weight (kg):

Selenium supplementation had highly significant ($P<0.01$) effect on weaning weight of piglets among treatments. Weaning weight was higher for T_3 piglets (5.93 ± 1.85kg) and it differed significantly ($P<0.05$) from those in T_0 (5.07 ± 5.92kg) T_1 (5.79 ± 0.02kg) and T_2 (5.76 ± 2.64kg). However, weaning weight between T_1 and T_2 was similar ($P>0.05$) but differed ($P<0.05$) from those in T_0.

4.2 Effect of Dietary Selenium Supplementation on Haematological Indices of Sows during Gestation and Lactation

4.2.1 Effect of dietary selenium supplementation on haematological indices of sows during Second trimester of gestation

The results of the effect of dietary selenium supplementation on haematological indices of sows during gestation are presented in Table 5 and 6.

TABLE 5: Effects of dietary selenium supplementation on haematological indices of sows during Second trimester gestation (Mean ± SE)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
PCV (%)	40.50 ± 2.50	42.00 ± 2.00	39.50± 0.50	38.10 ± 0.10	0.05^{NS}
Hb (%)	12.80 ± 1.10	12.85 ± 0.10	13.00 ± 0.40	13.75 ± 0.15	0.69^{NS}
RBC (10^7/l)	142.5 ± 37.5	215.0 ± 25.0	215.0 ± 25.0	197.0 ± 1.00	0.29^{NS}
WBC (10^9/l)	18.40 ± 5.60	14.40 ± 2.40	16.70 ± 2.10	17.35 ± 0.05	0.84^{NS}

[a,b,c] ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05), NS- Not significant

Pack Cell Volume (PCV):

Selenium supplementation had no significant effect (P>0.05) on PCV value among treatments. Sows in T_0 (40.50 ± 2.50%), T_1 (42.00 ± 2.00%), T_2 (39.50± 0.50%) and T_3 (38.10±0.10%) had similar PCV concentrations.

Haemoglobin (Hb): The effect of Selenium supplementation on Hb concentration was not significant (P>0.05) among treatments. Sows in T_0 (12.80 ± 1.10%), T_1 (12.85 ± 0.10%), T_2 (13.00 ± 0.40%) and T_3 (13.75 ± 0.15%) had similar Hb concentrations (P>0.05).

Red Blood Cell (RBC):

Selenium supplementation had no significant effect (P>0.05) in RBC counts among treatments. Sows on T_0 (12.80 ± 1.10(10^7/l)), T_1 (12.85 ± 0.10(10^7/l)), T_2 (13.00 ± 0.40(10^7/l)) and T_3 (13.75 ± 0.15(10^7/l)) had similar RBC concentrations (P>0.05).

White Blood Cell (WBC):

Selenium supplementation had no significant effect (P>0.05) on WBC concentration among treatments. Sows in T_0 (18.40 ± 5.60(10^9/l)), T_3 (17.35 ± 0.05(10^9/l)), T_2 (16.70 ± 2.10(10^9/l)) and T_1 (14.40 ± 2.40(10^9/l)) had similar WBC concentrations.

TABLE 6: Effects of dietary selenium supplementation on haematological indices of sows during Third trimester gestation (Mean ± SE)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
PCV (%)	32.50 ± 0.25^{b}	38.50 ± 0.08^{a}	37.00 ± 1.00^{a}	34.50 ± 0.50^{b}	0.03*
Hb (%)	11.40 ± 0.00	12.85 ± 0.15	12.25 ± 0.65	12.05 ± 0.35	0.20^{NS}
RBC (10^7/l)	131.50 ± 28.5^{C}	185.0 ± 35.0^{b}	177.5 ± 32.5^{b}	210.0 ± 20.0^{a}	0.04*
WBC (10^9/l)	16.00 ± 6.00	16.20 ± 3.00	12.40 ± 1.20	11.10 ± 0.30	0.67^{NS}

[a,b,c] ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05), NS- Not significant

Pack Cell Volume (PCV):

The effect of Selenium supplementation in diets of sows on PCV during 3rd trimester was significant (P<0.05) among treatments. Sows in T_1 (38.50 ± 0.08%) had higher PCV values among treatments but was comparable (P>0.05) with those in T_2 (37.00 ± 1.00%). However sows in T_0 (32.50 ± 0.25%) and T_3 (34.50 ± 0.50%) had similar PCV concentrations (P>0.05) which was lower than those of other treatments.

Haemoglobin (Hb):

Dietary Selenium supplementation had no significant effect (P>0.05) on Hb concentration in sow of various treatments. Sows in T_1 (12.85 ± 1.15%), T_2 (12.25 ± 0.65%), T_3 (12.05 ± 0.35%) and T_0 (11.40 ± 0.00%) had similar Hb concentrations.

Red Blood Cell (RBC):

The effect of Selenium supplementation was significant (P<0.05) among treatments on RBC counts. Sows in T_3 (210.0 ± 20.0) had higher (P<0.05) RBC counts which differed from those of sows in other treatments. However, Sows on T_1 (185.0 ± 35.0(10^7/l)) and T_2 (177.5 ± 32.5(10^7/l)) had similar (P>0.05) RBC counts but differed significantly (P<0.05) from those on T_3 (131.50 ± 28.5(10^7/l)) which has lowest RBC counts.

White Blood Cell (WBC):

Dietary Selenium supplementation had no significant effect (P>0.05) on WBC counts among treatments. Sows in T_0 (16.00 ± 6.00 (10^9/l)), T_1 (16.20 ± 3.00(10^9/l)), T_2 (12.40 ± 1.20(10^9/l)) and T_3 (11.10 ± 0.30(10^9/l)) had similar WBC concentrations.

TABLE 7: Effects of dietary selenium supplementation on haematological indices of sows during lactation (Mean ± SE)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
PCV (%)	39.00 ± 1.00	34.50 ± 2.50	35.50± 0.50	34.00± 4.00	0.385 ^{NS}
Hb (%)	10.75 ± 0.30	12.65 ± 0.55	11.90 ± 0.30	11.30 ± 1.40	0.548 ^{NS}
RBC (10^7/l)	108.0 ± 2.00^b	165.0± 15.0^a	175.0 ± 5.00^a	165.0± 5.00^a	0.015*
WBC(10^9/l)	22.07±0.44^a	11.20±0.80^b	9.00± 1.00^b	10.00±3.60^b	0.025*

^{a,b,c} ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05), NS- Not significant

Pack Cell Volume (PCV):

The effect of Selenium supplementation in the diets of sows on PCV during lactation was not significant (P>0.05) among treatments. Sows in T_0 (40.50 ± 2.50%), T_1 (42.00 ± 2.00%), T_2 (39.50± 0.50%) and T_3 (38.10±0.10%) had similar PCV concentrations (P>0.05).

Haemoglobin (Hb):

Dietary selenium supplementation had no significant effect (P>0.05) on Hb concentration in sows of various treatments. Sows in T_1 (12.65 ± 0.55%), T_2 (11.90 ± 0.30%), T_3 (11.30 ± 1.40%) and T_0 (10.75 ± 0.30%) had similar Hb concentrations.

Red Blood Cell (RBC):

Dietary selenium supplementation was significant (P<0.05) among treatments in RBC counts. Sows in T_2 (175.0 ± 5.00(10^7/l)), T_1 (165.0± 15.0(10^7/l)) and T_3 (165.0± 5.00(10^7/l)) had

similar (P>0.05) RBC counts which differed from those on T_0 (108.0 ± 2.00(10^7/l)) which had the lowest RBC counts.

White Blood Cell (WBC):

Selenium supplementation was significant (P<0.05) among treatments in WBC counts. Sows in T_0 (22.07±0.44 (10^9/l)) had high (P<0.05) WBC counts which differed from those of sows in other treatments. However, Sows on T_1 (11.20±0.80 (10^9/l)), T_2 (9.00± 1.00 (10^9/l)) and T_3 (10.00±3.60 (10^9/l)) had similar (P>0.05) WBC counts.

4.3 Effects of Dietary Selenium Supplementation on Selenium and Oxidative Enzyme Status of Sows during Gestation and Lactation

4.3.1 Effect of dietary selenium supplementation on selenium concentration and oxidative enzyme status of sows during gestation.

The effect of dietary selenium supplementation on oxidative enzymes status of sows during Second and Third trimester of gestation are presented in Tables 8 and 9, respectively.

Table 8: Effects of dietary selenium supplementation on selenium and oxidative enzyme status of sows during Second trimester of gestation (mean ± se)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
Selenium (u/ml)	0.91 ± 0.62^{ab}	1.63 ± 0.10^{a}	1.57 ± 0.05^{a}	0.24 ± 0.02^{b}	0.04*
Catalase (u/ml)	4.05 ± 0.17	4.17 ± 0.10	4.13 ± 0.06	3.91 ± 0.01	0.41^{NS}
SOD (u/ml)	0.94 ± 0.12^{a}	0.64 ± 0.02^{b}	0.54 ± 0.00^{b}	0.36 ± 0.02^{c}	0.01*
MDA (u/ml)	4.17 ± 2.66^{b}	0.34 ± 0.13^{c}	2.84 ± 0.38^{b}	7.17 ± 0.04^{a}	0.05*
Reductase (u/ml)	1.57 ± 0.11^{a}	1.33 ± 0.00^{b}	1.47 ± 0.01^{ab}	1.44 ± 0.01^{ab}	0.05*
GSH-Px (u/ml)	15.86 ± 2.22	16.92 ± 0.15	13.47 ± 0.14	14.29± 0.02	0.27^{NS}

^{a,b,c} ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05; ** P<0.01), NS- Not significant

Selenium (u/ml):

Dietary selenium supplementation had significant ($P < 0.05$) effect on blood Selenium concentration among treatments. Sows on T_1 (1.63 ± 0.10 u/ml) and T_2 (1.57 ± 0.05 u/ml) had ($P < 0.05$) higher Selenium concentration in the blood than other treatments. However there was no significant difference ($P > 0.05$) between Sows in T_1, T_2 and T_3 (0.91 ± 0.62 u/ml) as well as between Sows in T_0 and T_3 (0.24 ± 0.02u/ml).

Catalase (u/ml):

Selenium supplementation had no significant ($P<0.05$) effect on catalase concentration among treatments. Sows in T_0 (4.05 ± 0.17u/ml), T_1 (4.17 ± 0.10u/ml), T_2 (4.13 ± 0.06u/ml) and T_3 (3.91 ± 0.01u/ml) had similar catalase concentrations ($P>0.05$).

Superoxide Dismutase (SOD) (u/ml):

Selenium supplementation had significant ($P<0.05$) effect on SOD concentration among treatments. Sows in T_0 (0.94 ± 0.12u/ml) had significantly ($P<0.05$) higher SOD concentration than other treatments. However there was no significant difference ($P>0.05$) between sows in T_1 (0.64 ± 0.02 u/ml) and T_2 (0.54 ± 0.00u/ml) which had similar and comparable SOD concentrations but differed from sows in T_3 (0.36 ± 0.02u/ml) which had the least SOD concentration.

Malondialdehyde (MDA) (u/ml):

The effect of selenium supplementation on MDA concentration was significantly different ($P<0.05$) among treatments. Sows in T_3 (7.17 ± 0.04u/ml) had significantly ($P<0.05$) higher MDA concentration than other treatments. However there was no significant difference ($P>0.05$) between sows in T_0 (4.17 ± 2.66 u/ml) and T_2 (2.84 ± 0.38u/ml) which had similar MDA but differed significantly ($P<0.05$) from those in T_1 (0.34 ± 0.13 u/ml) which had the least MDA values.

Reductase (u/ml):

The effect of Selenium supplementation on reductase concentration was significantly (P<0.05) different among treatments. Sows in T_0 (1.57 ± 0.11u/ml) had significantly higher reductase concentration but was comparable with those of sows in T_2 and T_3. However there was no significant difference (P>0.05) between sows in T_2 (1.47 ± 0.01u/ml), T_3 (1.44 ± 0.01u/ml) and T_1 (1.33 ± 0.00u/ml) which had similar reductase concentrations.

Glutathione Peroxidase (GSH-Px) (u/ml):

Selenium supplementation had no significant (P<0.05) effect on GSH-Px concentration among treatments. Sows in T_1 (16.92 ± 0.15u/ml), T_0 (15.86 ± 2.22u/ml), T_2 (13.47 ± 0.14u/ml) and T_3 (14.29± 0.02u/ml) had similar GSH-Px concentrations (P>0.05) although they differed numerically.

SELENIUM SUPPLEMENTATION DURING THIRD TRIMESTER OF GESTATION

Table 9: Effects of dietary selenium supplementation on selenium and oxidative enzyme status of sows during Third trimester of gestation (mean ± se)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
Selenium (u/ml)	0.68 ± 0.23^{c}	2.09 ± 0.44^{a}	1.75 ± 0.16^{ab}	1.29 ± 0.02^{bc}	0.009*
Catalase (u/ml)	4.09 ± 0.12^{a}	3.95 ± 0.05^{a}	4.07 ± 0.29^{a}	2.95 ± 0.53^{b}	0.0164*
SOD (u/ml)	0.93 ± 0.07^{a}	0.47 ± 0.02^{b}	0.52 ± 0.01^{b}	0.48 ± 0.02^{b}	0.001 **
MDA (u/ml)	3.76 ± 1.74^{a}	0.12 ± 0.03^{b}	0.11 ± 0.35^{b}	4.36 ± 0.62^{a}	0.004**
Reductase (u/ml)	0.59 ± 0.04^{b}	1.46 ± 0.04^{a}	1.46 ± 0.00^{a}	1.48 ± 0.02^{a}	0.046*
GSH-Px (u/ml)	15.00 ± 0.79	14.24 ± 0.69	16.06 ± 0.71	14.75±0.41	0.284^{NS}

^{a,b,c} ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05; ** P<0.01), NS-Not significant

Selenium (u/ml):

Selenium supplementation on blood selenium concentration in sows was significant (P<0.05) among treatments. Sows on T_1 (2.090 ± 0.44 u/ml) and T_2 (1.747 ± 0.162 u/ml) had

significantly (P<0.05) higher Selenium than other treatments. However there was no significant difference (P>0.05) in blood selenium between Sows in T_2 and T_3 (1.285 ± 0.020 u/ml) as well as between Sows in T_3 and T_0 (0.679 ± 0.225 u/ml).

Catalase (u/ml):

Selenium supplementation had significant (P < 0.05) effect on Catalase concentration among treatments. Sows on T_0 (4.09 ± 0.12 u/ml) and T_2 (4.07 ± 0.29 u/ml) had higher but similar catalase concentration which differed (P<0.05) from those of other treatments. Catalase concentration of sows in T_1 (0.472 ± 0.02 u/ml) and T_3 (2.950 ± 0.53 u/ml) were similar (P>0.05).

Superoxide Dismutase (SOD) (u/ml):

Selenium supplementation had highly significant (P<0.01) effect on SOD concentration among treatments. Sows in T_0 (0.93 ± 0.07 u/ml) had significantly (P<0.05) higher catalase concentration than other treatments. However there was no significant difference (P>0.05) between sows in T_1 (0.47 ± 0.02 u/ml), T_2 (0.52 ± 0.01 u/ml) and T_3 (0.48± 0.02 u/ml) which had similar SOD concentrations.

Malondialdehyde (MDA) (u/ml): Selenium supplementation on MDA concentration was significant (P<0.05) among treatments. Sows in T_3 (4.36 ± 0.62 u/ml) had significantly (P<0.05) higher MDA concentration than other treatments but was similar (P>0.05) with sows in T_0 (3.76 ± 1.74 u/ml). However there was no significant difference (P>0.05) between sows in T_1 (0.12 ± 0.03u/ml), T_2 (0.11±0.35 u/ml) which had similar MDA concentrations.

Reductase (u/ml):

Selenium supplementation on reductase concentration was significant (P<0.05) among treatments. Sows in T_0 (0.59 ± 0.04 u/ml) had significantly (P<0.05) lower reductase concentration than other treatments. However there was no significant difference (P>0.05)

between sows in T_1 (1.46 ± 0.04u/ml), T_2 (1.46 ± 0.00 u/ml) and T_3 (1.48 ± 0.02 u/ml) which had similar reductase concentrations.

Glutathione Peroxidase (GSH-Px) (u/ml):

Selenium supplementation had no significant (P>0.05) effect on GSH-Px concentration among treatments. Sows in T_0 (15.00 ± 0.79u/ml), T_1 (14.24 ± 0.69u/ml), T_2 (16.06 ± 0.71u/ml) and T_3 (14.75±0.41u/ml) had similar GSH-Px concentrations (P>0.05) although they differed numerically.

4.3.2 Effect of dietary selenium supplementation on selenium concentration and oxidative enzyme status of sows during lactation

The effect of dietary selenium supplementation on oxidative enzymes status of sows during lactation is presented in Tables 10

Table10: Effect of Dietary Selenium Supplementation on Selenium and Oxidative Enzyme Status of Sows during Lactation (MEAN ± SE)

	Treatment groups				
Parameters	T_0	T_1	T_2	T_3	SEM
Selenium (u/ml)	0.27 ± 0.23^{b}	2.20 ± 0.21^{a}	2.55 ± 0.01^{a}	1.29 ± 0.08^{a}	0.001 **
Catalase (u/ml)	3.89 ± 0.01^{a}	3.25 ± 0.06^{b}	3.30 ± 0.08^{b}	3.27 ± 0.12^{b}	0.001**
SOD (u/ml)	0.46 ± 0.07	0.41 ± 0.01	0.46 ± 0.01	0.38 ± 0.01	0.376^{NS}
MDA (u/ml)	7.26 ± 0.08^{a}	3.60 ± 0.22^{b}	3.70 ± 0.27^{b}	2.27 ± 0.11^{c}	0.001**
Reductase (u/ml)	0.61 ± 0.03^{c}	1.51 ± 0.00^{a}	1.51 ± 0.00^{a}	1.48 ± 0.03^{b}	0.001**
GSH-Px (u/ml)	13.26 ± 0.10	12.98 ± 0.07	12.58 ± 0.56	14.75± 0.18	0.213^{NS}

^{a,b,c} ï Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05; ** P<0.01), NS-Not significant

Selenium (u/ml):

Selenium supplementation was highly significant (P< 0.01) on Selenium concentration of Sows. Sows in T_0 (0.269 ± 0.249 u/ml) had higher (P< 0.01) Selenium concentration than Sows in other treatments. However, Sows in T_1 (2.20 ± 0.21u/ml), T_2 (2.55 ± 0.01u/ml) and

T_3 (1.29 ± 0.08u/ml) had similar Selenium concentrations. Results showed higher selenium concentration in blood of sows fed dietary selenium (T_1, T_2, T_3) compared to control treatment (T_0).

Catalase (u/ml):

Selenium supplementation had highly significant ($P<0.01$) effect on Catalase concentration among treatments. Sows in T_0 (3.89 ± 0.01u/ml) had higher catalase concentration which differed ($P<0.01$) from those of other treatments. However, sows on T_1 (3.25 ± 0.06 u/ml) T_2 (3.30 ± 0.08 u/ml) and T_3 (3.27 ± 0.12 u/ml) had similar Catalase concentration ($P>0.05$).

Superoxide Dismutase (SOD) (u/ml):

Selenium supplementation had no significant ($P>0.05$) effect on SOD concentration among treatments. Sows in T_0 (0.46 ± 0.07u/ml), T_1 (0.41 ± 0.01u/ml), T_2 (0.46 ± 0.01 u/ml) and T_3 (0.38 ± 0.01u/ml) had similar SOD concentrations ($P>0.05$).

Malondialdehyde (MDA) (u/ml):

Selenium supplementation had highly significant ($P<0.01$) effect on MDA concentration of Sows. Sows on T_0 (7.26 ± 0.08 u/ml) had significantly ($P<0.01$) higher MDA concentration than sows in other treatments. However, Sows in T_1 (3.60 ± 0.22 u/ml) and T_2 (3.70 ± 0.27 u/ml) had similar MDA concentrations which differed significantly ($P<0.05$) from those in T_3 (2.27 ± 0.11 u/ml) which had the lowest mean MDA concentration.

Reductase (u/ml):

The effect of selenium supplementation was significant on reductase concentration of Sows among treatments. Sows in T_0 (0.61 ± 0.03u/ml) had highly significantly ($P<0.01$) different reductase concentration than Sows in other treatments. However, Sows in T_1 (1.51 ± 0.00 u/ml) and T_2 (1.51 ± 0.00 u/ml) had similar ($P>0.05$) reductase concentration which differed ($P<0.05$) from those of Sows in T_3 (1.423 ± 0.032 u/ml) with the lowest reductase mean

concentration. The mean reductase concentrations of sows in the treatments declined gradually across the supplemented group

Glutathione Peroxidase (GSH-Px) (u/ml):

There were no significant effect (P>0.05) of selenium supplementation on GSH-Px concentration of sows in the various treatments. Sows in T_0 (13.26 ± 0.10u/ml), T_1 (12.98 ± 0.07u/ml), T_2 (12.58 ± 0.56u/ml) and T_3 (14.75± 0.18u/ml) had similar GSH-Px concentrations (P>0.05).

DISCUSSION

4.4.1. Effect of Dietary Selenium Supplementation on Sow Reproductive Performance and Preweaning Growth Performance of Piglets

Effect of supplementing sows diet with organic selenium had no significant effect (P>0.05) on sow body weight during gestation, sow body weight at farrowing, sow gestation weight gain, sow body weight loss during lactation and litter size at birth. This finding is in agreement with the reports of Mahan and Kim (1996) who reported that Se source and supplementation had no effect on gilts reproductive performance. It also agrees with the findings of Hamed (2005), who reported non insignificant effect of selenium levels on gestation length in New Zealand white does. Gestation length is genetically determined and does not change much in animals. Similar findings by Hasan and Owolabi (1996) showed that nutrition had no effect on gestation length although it can be affected by litter size. Svoboda *et al.* (2008 and 2009) also reported that the number of piglets born (total, live) was not affected by selenium supplementation. Selenium supplementation had significant effect (P<0.05) on litter size at weaning. Litter size at weaning was higher in T_3 (7.86 ± 0.67kg) compared to the control group T_0 (3.33±0.33kg). Generally, piglets of sows fed organic selenium during gestation and lactation had better results compared to the control. The weaning weight was significantly higher in T_3 (5.93±1.85kg) than in the control group T_0 (5.07±5.92kg). A considerable decrease in mortality was seen in the selenium treated groups especially in T_3 (0.47±3.05%) than in the control T_0 (2.33±0.33%). These findings are in tune with the results of Stanley (2001) who reported that selenium supplementation resulted to higher survival rate and better growth of piglets. This improvement may be attributed or seen as an indication that selenium during gestation and lactation alleviated oxidative stress on the sows which led to improvement in the sowôs ability to produce sufficient amount of milk without much stress. This in turn increased the vitality of the piglets due to better transport of

selenium and other nutrients from mother to offspring through the milk and also elevated the immunity of piglets and reduced piglet mortality (Stanley, 2001).

4.4.2 Effect of dietary selenium supplementation on preweaning growth performance of piglets

Results showed that selenium supplementation in diets of sows during gestation and lactation had no significant effect (P>0.05) on piglet birth weight. This finding is in agreement with the reports of Mahan. (2000); Yoon and Mcmillian (2006) who reported no differences in litter size at birth, piglet birth weight when dietary selenium was supplemented during gestation. However, this result is not in agreement with the reports of Mahan (1991), Mavromatis *et al.* (1999), Migdal and Kackzmarczyk (1993) who found improvements in piglet birth weight and litter size when sows diet during gestation and lactation was supplemented with Vitamin E and 0.30 mgSe via injection compared to control animals. This differences in results may be due to the source of selenium, the longer duration of the treatment (throughout gestation) and also due to the synergistic effect of selenium and Vitamin E in boosting reproduction.

Selenium supplementation elicited no significant effect (P>0.05) on piglet weight gain between the control group and the treated group during the first two (2) weeks of age. However, there were significant differences in weight gain from the 3rd week to weaning. This increase in weight gain from the 3rd week coincides with the period of improved milk production in sows which usually peaks around day 21 (Aherne, 2007). Also significant differences were seen in pre weaning weight gain and weaning weights of piglets among treatments. This result is in agreement with the results of Pineda *et al.* (2004) who reported improvement in piglets weaning weight when sows diet was supplemented with selenium yeast during gestation and lactation. This is because of better transport of selenium and other

nutrients from mother to offspring through milk which is of benefit to the neonate and this elevated the weight gain and immunity of piglets.

4.4.3. Effect of Dietary Selenium Supplementation on Haematological Indices of Sows during Gestation and Lactation

Effects of supplementing sows diet with organic selenium on Hb concentration were not significant (P>0.05) at gestation and lactation. This result is in agreement with the reports of Kooshiki *et al*. (2010) who reported that selenium supplementation did not change Hb compared with control group irrespective of the numerical increase in Hb (g/dl) values in the Se supplemented groups. On the other hand, selenium supplementation had significant (P<0.05) effect on PCV percentage during gestation but was not significant during the lactation phase. This shows that physiological status of the animal had effect on the PCV value. Selenium supplementation decreased PCV % values in the supplemented group. The lowest values was recorded in sows in T_3 (34.50±0.50%) and T_2 (37.00±1.00%) during the third trimester of gestation. A decrease in PVC below normal values in healthy pigs indicates the presence of anaemia, vitamin or mineral deficiencies (Mercks manual, 2012). Irrespective of the decline in PCV values during the third trimester which is as a result of increased physiological stress in the Se supplemented groups it was still within the normal PCV range for healthy pigs (33-43%) as reported by Mercks manual (2012). This report is in agreement with the result of Abdel, (2010) who found a decrease in PCV values of rabbits with Se supplementation than without Se supplementation. The author stated that the decline may be due to the small size of the RBCs.

Organic Selenium supplementation in sows diet during gestation and lactation elicited significant difference (P<0.05) in RBC concentration. RBC counts were found to be significantly higher in sows fed diet supplemented with Se compared with those in the control diet which had the lowest values (131.50±28.50 (10^7/l) and 108.0±2.0 (10^7/l)) during third

trimester of gestation and lactation respectively. This finding is in agreement with the reports of Chen and Lin (2000), Faixova *et al.* (2007), Ahmed (2010) and Chen and Lin (2000) who reported a positive role of Se on haematological indicators in rat, calves, ewe and male broilers. Physiological status of the sow had significant effect on RBCs count. The RBC count was high during gestation but however, decreased during lactation. According to Rechcigl (1994), this reduction in RBC is associated with the stress of farrowing and nursing which happens in all animals, but the decrease was most pronounced in the control group indicating the important role of Se supplementation in alleviating sow stress during gestation and lactation.

Effect of supplementation of sows diet with organic selenium had significant ($P<0.05$) effect on WBC counts. According to Entrican *et al.* (2002), the development of an embryo in the maternal organism is accompanied by a higher stress on the immune system as evidenced in the establishment of motherôs immunotolerance to the embryoôs extraneous antigens. During the second trimester of gestation there was an increase in WBC counts in Se treated groups. However, this increase had no significant effect compared with other treatments. This slight increase could be attributed to the stress associated with pregnancy which may have been suppressed by selenium supplementation in the sow diet. The significant decrease in WBC counts seen during the lactation was as a result of the boost in immunity conferred to the sow through the antioxidative property of selenium. The decrease in WBC counts in the selenium supplemented groups of sows observed in the study is in agreement with the finding of Faxiova *et al*. (2007) who reported decrease WBC counts in sheep fed diet supplemented with 0.30mgSe/kg diet compared with the control group.

As observed in the study (Tables 6 and 7), the physiological status of the sow had effect on WBCs concentrations. These results indicated that WBCs counts were increased during pregnancy, while decreased in lactation phase. This increase during gestation is due to

the desperate need to increase immunity which the sow passes to offspring through the milk. Similarly, a significant increase of WBCs count in pregnant ewes has been reported by Kim *et al*. (2000), who found that WBCs count increased in pregnant rats.

4.4.4. Effects of Dietary Selenium Supplementation on Selenium and Oxidative Enzyme Status of Sows During Gestation and Lactation

The results of the study showed that selenium supplementation had significant impact ($P<0.05$) in blood selenium concentration among the various treatments. Selenium is an essential trace element for maintaining normal physiological process in animals. It exerts multiple actions on the antioxidant (Arthur, 2000; Tapiero *et al.,* 2003) and immune systems (Beck *et al.,* 2005). Blood Se concentration increased in the supplemented group than in the control group. This finding agrees with the results of Adkin and Ewan, (1984), Mahan *et al.* (1999) and Tian *et al.,* (2006). These authors in their various studies noted that that blood Se concentration increased with Se supplementation in pig. Similar findings have been reported in broilers (Yoon, *et al.,* 2007; Ryu *et al.,* 2005), beef (Beck, *et al.,* 2005) and sheep (Qin *et al.,* 2007).

The result of the study showed that selenium supplementation had significant ($P<0.05$) influence in catalase activity among sows in the various treatments. In the second trimester of gestation, selenium supplementation had no significant effect in catalase activity among the various treatments. During gestation (third trimester), it was observed that selenium supplementation increased catalase activity among the treated groups and the control group except T_3 (lactation) which had the lowest value (2.95±0.53µ/ml). During the lactation phase it was found that catalase activity was higher in the control group (3.89±0.01 µ/ml) while the treated groups has similar catalase values of 3.25 ± 0.06 µ/ml, 3.30 ± 0.08 µ/ml and 3.27 ± 0.12 µ/ml for T_1,T_2 and T_3, respectively. In the cell, catalase reacts with generated hydrogen peroxide to form water and molecular oxygen thereby protects the cell

against hydrogen peroxide toxicity and lipid peroxidation (Yamaguchy, 1991). Milinkoric-Tur *et al.* (2009) reported that catalase activity as well as other anti oxidant enzymes activity depends on the presence of other antioxidant (selenium, vitamin E, etc) in the diet. The present study disagrees with the finding of these authors because the highest means catalase values were observed in the control groups. This may probably be because the duration of selenium supplementation was short to elicit its antioxidant effect compared to the case of Milinkoric- Tur *et al.* (2009) who supplemented selenium in form of selenoyeast throughout the production cycle.

Superoxide dismutase (SOD) is an enzyme that catalyses the dismutation of superoxide (O^-_2) to oxygen and hydrogen peroxide. Therefore they are important antioxidant defence in nearly all cells exposed to oxygen (Yamaguchy, 1991). Effect of selenium supplementation showed non-significant difference ($P>0.05$) in SOD concentration among treatments during the lactation phase. This may be that the level of selenium administered at the lactation stage was not enough to calm the oxidative stress in the Se treated group. However, there was a highly ($P<0.01$) significant difference in SOD concentration during gestation (2^{nd} and 3^{rd} trimester) with the highest mean SOD values being observed in the control groups (0.94 ± 0.12 and 0.93 ± 0.07 µ/ml) during the second and third trimester respectively while it was similar among the treated groups. Although there was significant difference, it did not agree with the reports of Gao *et al.* (2006) who reported higher plasma SOD activity in pigs supplemented with Se probiotics than those in the control group. These conflicting results may be attributed to the duration the animals received the selenium. In the case of Gao *et al.* 2006 the animals were fed Selenium for longer duration (60 days). The low SOD in the Se treated group may result from higher activity of the SOD enzymes in catalysing the dismutation of superoxide (O^-_2) to oxygen and hydrogen peroxide in the system thereby leading to its low level in the body.

Malondialdehyde (MDA) is an indicator or marker of lipid peroxidation. Lipid peroxidation is the product of oxidative damage and plays an important role in oxidative stress (Dzobo and Yogeshkumar, 2011). In the present study, there was significant ($P<0.05$) effect in malondialdehyde levels in the blood of the Se supplemented group compared to the control group during gestation and lactation phases. It was observed that the highest MDA was observed in the non supplemented/ non treated groups at both stages; gestation and lactation (7.26±0.08µ/ml) while the treated group had lower MDA levels at both stages. During the second trimester, the highest values were recorded in the untreated groups, T_0, T_2 and T_3. In the third trimester, MDA values was also highest in the untreated groups T_0 and T_3 but was lowest in the supplemented or treated groups. These results show a positive role of selenium in combating or reducing oxidative stress which was seen in decreased lipid formation. Similar findings were also reported by Sahin *et al.* (2009) and Zhang *et al.* (2009) showing that supplementing antioxidant can reduce the formation of lipid peroxides under heat stress condition in broilers and goats, respectively. Reduction in MDA concentration in the present study suggests that Se supplementation improved the antioxidant status in pigs. Based on the results, 0.30mgSe/kg diet can improve antioxidant status in pigs and it is in agreement with the findings of Gao *et al.* (2006) who reported that MDA concentration in pigs decreased with Se probiotic supplementation.

Glutathione reductase is a key enzyme of the anti oxidant system that protects cells against free radicals. It catalyses the reduction of glutathione disulfide (GSSG) to sulfhydryl form GSH by the NADPH-dependent mechanism (Berivan and Nuray 2006). Decreased GSH/CSSG ratio contributes to oxidative stress. In the present study, it was observed that the treated groups had significantly ($P<0.01$) higher Glutathione reductase concentration during gestation and lactation compared to the control group. This result is in agreement with the reports of Hu *et al.* (2011) and Zhan *et al.* (2006) who reported that the supplementation of

0.30mg Se/kg in organic form leads to a significant increase in Glutathione reductase concentration in sow blood and milk.

Glutathione peroxidase enzymes (GSH-Px) are antioxidant enzymes that catalyze the reduction of hydrogen peroxide and lipid hydroperoxides to destroy free radicals produced during normal metabolic activity. These enzymes catalyze a reaction that removes hydrogen peroxide from erythrocytes via reduced glutathione. The reduced glutathione is made via the enzyme, glutathione reductase, from oxidized glutathione (Payne, 2004). In the present study there was no significant difference ($P > 0.05$) in the activity of GSH ï Px among treatments. This finding is not in agreement with the previous reports in which Se supplementation increase plasma GSH ï Px activity in broilers (Canter *et al.,* 1982, Yoon *et al.,* 2007, Pavel *et al.,* 2013), pigs (Adkin and Ewan, 1984; Mahan, *et al.* 1999) and sheep (Qin *et al.,* 2007). However, it agrees with the reports by Cantor *et al.,* 1975 and Payne and Southern 2005, Svoboda *et al.,* 2008 who reported lack of response in plasma GSH ï Px activity to Se supplementation in chicks and broilers. This lack of response may have been due to either the levels of Se supplemented, source of selenium (organic or inorganic) or the concentration of Se in the basal diet. In the present study, the duration of selenium supplementation was set at 30 days which probably did not influence a resultant increase in GSH-Px concentration. Pavel *et al., (*2013) supplemented selenium at 0.30mgSe/ kg diet and 0.55mgSe kg diet of sows for 8 weeks post partum period.

Conclusion and Recommendation

In the study which was based on supplementation of selenium in organic form, its effect on the reproductive performance and antioxidant status of pregnant and lactating sows was monitored. From the results, 0.30mgSe/kg diet during gestation and lactation significantly improved the reproductive performance and antioxidant status of sows. The results showed that 0.30mgSe/kg diet supplementation during lactation had better results in

the reproductive performance and preweaning growth performance of their piglets compared to other treatments. Selenium was significantly increased in the erythrocytes of sows supplemented with selenium than those in the control group. The antioxidant status of sows was greatly improved in the Se supplemented group with sows in T_1 (2^{nd} trimester) having the best result. Summarily, supplementation with organic selenium improved reproductive performance, preweaning growth performance of their piglets and reduced oxidative stress in sows during the stressful period of gestation and lactation.

Based on the results of the study, it is therefore recommended that

i. 0.30mgSe/kg diet should be supplemented in the diet of sows during lactation for improved reproductive performance and preweaning growth performance of their piglets.

ii. For improved antioxidant status, 0.30 mgSe/kg diet should be supplemented during the 2^{nd} trimester of gestation.

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