Selenium × zinc interaction on growth performance, carcass traits and semen quality of Large White × Landrace and Kolbroek boars

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By

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Declaration

I, Thivhilaheli Richard Netshirovha, declare that this dissertation which I have complied and submitted to the University of KwaZulu-Natal for the PhD degree, represents my own work and has never been submitted to any tertiary institute for any degree.

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List of abbreviations

ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
AI	Artificial insemination
ALH	Amplitude of lateral head
ARC	Agricultural Research Council
AS	Abnormal spermatozoa
ATP	Adenosine triphosphate
BCF	Frequency with which the spermatozoa track crossed the spermatozoa path
BTS	Beltsville thaw solution
BW	Body weight
Ca	Calcium
CASA	Computer assisted semen analysis
CCW	Cold carcass weight
CL	Carcass length
cm	Centimetre
Co	Cobalt
CP	Crude protein
Cr	Chromium
Cu	Copper
DE	Duration of ejaculation
DFI	Daily feed intake
DM	Dry matter
DNA	Deoxyribonucleic acid
DS	Dead spermatozoa
EAA	Essential amino acids
EE	Ether extract
ELISA	Enzyme-linked immunosorbent assay
EV	Ejaculation volume
FCR	Feed conversion ratio

FSH	Follicle stimulating hormones
g	Gram
GCRB	Germplasm, Conservation and Reproductive Biotechnologies Unit
GSH-PX	Glutathione peroxidase
HOST	Hypo-osmotic swelling test
HQC	Hindquarter circumference
HQL	Hind quarter length
HQW	Hindquarter weight
HQWP	Hindquarter weight proportion
HSHZ	High selenium high zinc
HSLZ	High selenium low zinc
I	Iodine
IU	International Unit
K	Potassium
KB	Kolbroek
kg	Kilogram
LH	Luteinizing hormones
LP	Lipid peroxidation
LS	Live spermatozoa
LSHZ	Low selenium high zinc
LSLZ	Low selenium low zinc
LW	Large White
MDA	Malondialdehyde
ME	Metabolizable energy
mg	Milligram
Mg	Magnesium
mL	Millilitre
mm	Millimetre
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NDF	Neutral detergent fibre

NM	Number of mounts
NPM	Non-progressive motility
NRC	National Research Council
NS	Not significant
P	Probability
PM	Progressive motility
PSE	Pale, Soft, Exudative meat,
PUFAs	Peroxidised polyunsaturated fatty acids
REL	Right epididymis length
REW	Right epididymis weight
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Right testis
RTL	Right testis length
RTW	Right testis width
RW	Rib weight
RWP	Rib weight proportion
s.d	Standard deviation
SAS	Statistical analysis system
SC	Sertoli cell
SC	Spermatozoa concentration
Se	Selenium
SEM	Standard error of mean
SeMet	Selenomethionine
SOD	Superoxide dismutase
STR	Straightness
SV	Semen volume
SWP	Shoulder weight proportion
TBA	Thiobartituric acid
TFI	Total feed intake
TL	Testis length
TM	Total motility
TMNP	Time mounts with penis exposed

TMWP	Time mount without penis exposed
TSC	Total spermatozoa counts
TSE	Total spermatozoa in ejaculate
TW	Testis width
VAP	Velocity average pathway
VSL	Velocity curvilinear
WCW	Warm carcass weight
Zn	Zinc

Thesis outputs

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Interaction of selenium × zinc supplementation on testicular and accessory sex gland morphology and spermatogenesis in Large White × Landrace and Kolbroek boars. Animal Reproduction Science.

General abstract

This study evaluated the effect, in Kolbroek and Large White × Landrace (LW × LR) boars, of dietary supplemention with selenium and zinc on growth performance, fertility as determined by semen quality, epididymal spermatozoa quality, seminal plasma constituents, testicular morphology and histology, as well as on carcass traits and visceral macromorphometry. In Experiment 1, evaluated the effects of the interaction among genotype, selenium and zinc on growth performance of LW × LR and Kolbroek boars. 24 LW × LR and 24 Kolbroek boars, 7 to 8-months of age with an average body weight of 41.2 and 55 kg, respectively, were used. The boars were assigned to four experimental groups in a 2 × 2 × 2 factorial arrangement with six boars per treatment combination where in dietary treatments were: high selenium high zinc (HSHZ), high selenium low zinc (HSLZ), low selenium high zinc (LSHZ) and low selenium low zinc (LSLZ) inclusion in the feed. The pigs were fed their respective diet treatments for six months. There were genotype effects on average daily feed intake (ADFI, $P<0.05$ with LW x LR genotype having higher/lower ADFI. Dietary supplementation with Se and Zn had no effect on average daily gain (ADG). There was an increase of selenium and zinc supplementation, while the feed conversion ratio (FCR) increased in both genotypes ($P<0.05$). In conclusion, while pig genotype influenced growth performance; dietary supplementation with Se and or Zn did not affect growth performance.

In Experiment 2, assessed the effects of genotype × selenium × zinc on sexual behaviour, ejaculation semen volume and testosterone concentration in LW × LR and Kolbroek boars. Enzyme-linked immune sorbent assay (ELISA) technique was used to quantify plasma testosterone concentration. There was a genotype effect on time mounts with penis exposed (TMNP) and DE (duration of ejaculation) ($P<0.05$). Kolbroek boars, had increased the TMNP and DE values ($P<0.05$) than LW × LR boars. Kolbroek boars fed on HSHZ diet had lower

TMNP ($P < 0.05$) than those fed on the HSLZ diet. The LW × LR boars fed LSLZ diet had higher DE ($P < 0.05$) than boars fed on LSHZ and HSHZ diets. The LW × LR boars fed on HSHZ and HSLZ diets had higher ejaculation volume (EV) and testosterone concentrations than LW × LR boars fed on LSHZ and LSLZ diets. There were no differences in ejaculation volume and testosterone concentrations among the Kolbroek fed the different diets ($P>0.05$). In conclusion, selenium and zinc supplementation did not have any effects on testosterone production and DE. The was genotype effects on sexual behaviour, TMNP and testosterone concentration, with crossbred pigs producing higher semen volume than Kolbroek pigs.

In Experiment 3, assessed the effects of interaction of genotype, selenium and zinc supplementation on carcass characteristics and visceral macro-morphometry were assessed. Selenium and zinc supplementation did not influence viscera macro-morphometry and carcass traits and primal pork cuts in both genotypes ($P>0.05$). This implies that selenium and zinc supplementation may not be essential mineral in influencing growth performance of pigs. There were increased the weight of visceral organ weight indices and carcass traits and primal pork cuts in LW × LR pigs ($P<0.05$), implying that selenium and zinc have a greater role physiologically.

In Experiment 4, assessed the effects of selenium and zinc interaction on epididymis spermatozoa quality, seminal plasma constituents and lipid peroxidation in LW × LR and Kolbroek boars. Spermatozoa viability, lipid peroxidation and biochemical protein seminal plasma were analysed. There were no effects of genotype on macroscopic semen evaluation ($P > 0.05$). There was no influence of genotype, selenium and zinc supplementation on semen volume and spermatozoa concentration, distal droplets, midpiece, tails, spermatozoa motility

and velocity, seminal plasmaconstituents and hyper osmotic swelling test (P > 0.05). However, selenium and zinc supplementation improved spermatozoa quality and velocity and constituents of seminal plasma.

In Experiment 5, assessed the effects of interaction of selenium and zinc supplementation on testicular and accessory sex gland morphology and spermatogenesis. Testes were dissected, weighed. Widths, circumferences, morphology and spermatogenesis were evaluated. There were no changes in left and right testes weight indices in Kolbroek boars as selenium and zinc levels were increased (P > 0.05), while the indices decreased in LW × LR boars (P < 0.05). There was no influence of selenium and zinc supplementation on testicular and epididymis lengths and weights thickness germinal epithelium (P >0.05) in both genotypes. There were no effects of genotype, selenium and zinc on seminiferous tubule area, density of spermatogonia, Sertoli nuclear volume and density of Leydig cells (P >0.05). In conclusion, selenium and zinc levels improved accessory sex glands morphology.

Key words: crossbreed pigs, indigenous pigs, mineral, carcass traits, reproduction and growth traits blood metabolites, libido, testosterone.

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Chapter 1

General Introduction

1.1 Background

In rural communities, indigenous pigs are used as a source of income and can be used to generate and accumulate capital and productive investments (Chimonyo *et al*., 2005). However, little effort has been made to improve the marketing of local pigs (Hoffman *et al.,* 2005). It is important to conserve indigenous pig genotypes because of their diverse roles and functions in sustainable agricultural development (Chimonyo *et al*., 2005). The Kolbroek is a South African indigenous pig genotype with unique genetic traits for disease tolerance and adaptability in harsh environmental conditions (Masenya *et al.,* 2011). However, it deposits excess subcutaneous fat (Ramsay *et al*., 1994). Exotic pig genotypes have higher feed conversion efficiency and have higher growth potential as compared to indigenous pig genotypes (Ramesh *et al.,* 2009). Indigenous pigs contribute to human nutrition, food security, poverty allevation, and creation of employment for the rural communities.

Testicular size is a good predictor of semen production (Rathje *et al.,*1995; Huang and Johnson, 1996). Lowered fertility and/or lowered total spermatozoa counts (TSC) and decreased ejaculate volume have been found in boars during or shortly after the summer period (Einarsson *et al*., 2008). Thus, a better evaluation of the quality of the semen of each boar is imperative. The examination should provide a reliable evaluation of the donor. Furthermore, seminal quality evaluation can help identify causes of low fertility (Petrocelli *et al*., 2015). Improvement of productive traits in pigs is important in increasing profitability of pig enterprises. However, the improvement of productive traits ignoring reproductive traits cause

poor genetic progress (Rydhmer *et al*., 1995). Because of the poor genetic correlation between many production and reproduction traits, aselection index considering both should be used to improved populations (Oh *et al.*, 2006).

Due to the large demand for selenium and zinc, it is likely that bone mineral loss occurs in sows during gestation, particularly at late gestation and prior to lactation when the demands for calcium and phosphorous are higher. It is important for its role in regulating growth and development of the fetus and newborn and concentration levels are critical as both low and high levels have harmful manifestations (Jariwala et al., 2014). Similarly, the demand for calcium for milk production during lactation requires the dam to employ some form of adaptive mechanisms to provide sufficient calcium and phosphorous in the milk, while maintaining the homeostatic regulation of these minerals in her own body. Kovacs (2005) suggested that in humans, the extent of the different adaptive mechanisms to meet the demands for calcium and phosphorous differ between gestation and lactation.

Semen collections are negatively correlated with the reproductive performance of boars, whereas food supplementation, social contact with other pigs and the accuracy of semen processing protocols are positively correlated with artificial insemination outcomes (Hafez, 1993). Kolbroek, which is threatened with extinction, represent both national value and tremendous economic and genetic potential. Thus, their preservation needs the comprehensive collaboration of commercial and governmental actors as well as researchers (Rátky *et al.*, 2013). Various commercial forms of selenium found their way to the market place and was shown to be effective sources of selenium for poultry and animal production (Surai and Fisinin, 2015). In the last few decades, the commercial production of organic selenium from yeast has been developed (Surai, 2006). To enhance the contribution of indigenous pigs to the national

economy, new markets should be established. The limitations of using inorganic selenium are well known: toxicity, interactions with other minerals, low efficiency of transfer to milk, meat and eggs and inability to build and maintain selenium reserves in the body (Surai, 2006). Their existence would assist in maintaining genotype diversity and improve the livelihood of farmers (Egerszegi *et al.*, 2009). Zinc is an essential nutrient and indispensable element in growth and reproduction (Kumar *et al.*, 2014). Low selenium diets cause the mitochondria in the tail midpiece to become more oval with wider gaps between organelles. In addition, the plasma membrane connection to the tail midpiece becomes loosely bound (Surai *et al.*, 2015). The effect of selenium and zinc supplementation on semen quality of South African pig genotypesis not well documented.

Selenium and zinc prevent lipid peroxidation (LP) and stabilize lysosomal membrane (Kimball *et al.*, 1995) and hence improve fertility (Bray and Bettger, 1995; Kumar *et al.*, 2014). Combination of supplementation of selenium and zinc produce more efficient protection to spermatozoa (Said *et al.*, 2010). Selenium and zinc act as cofactors in the synthesis of anti-oxidative enzymes, superoxide dismutase and glutathione peroxidase. Selenium level in blood is also positively correlated with acrosome integrity (Bertelsmann *et al.*, 2007; Horky *et al.*, 2012). Selenium is an essential part of a family of enzymes called glutathione peroxidases (GSH-Px) and thioredoxin reductases which are important for neutralizing free radicals (Yatoo *et al*., 2013). Selenium is important for the production and maturation of spermatozoa (Marin-Guzman *et al.*, 2000; Lasota *et al.*, 2004) and improves semen quality (Kołodziej and Jacyno, 2005). Zinc is involved in the secretion and function of testosterone through the enzymes that control the arachidonic acid cascade. Zinc plays an essential role in sexual development and spermatogenesis (Yatoo *et al.*, 2013). Currently, no information is available on zinc and

selenium supplementation and their interaction on growth, and reproduction performance, sexual behaviour, testosterone hormones, carcass characteristics and blood metabolites of Kolbroek and LW × LR boars.

1.2 Justification

An unfortified animal diet is low in zinc to meet daily requirements (Hassan, 2009). Zinc and selenium are essential micro-minerals that optimise growth and reproduction and stimulate immune responses in pigs (Kumar *et al*., 2014). Selenium supplementation in growing/finishing pigs increases muscle tissue (Close and Cole, 2001). Supplementation of selenium fed in the boar seems to affect the metabolic activity of spermatozoa in the ejaculate and thus a deficiency could contribute to a low motility and fertilization rate in sows (Kamel, 2012). Surai (2006) suggested that antioxidant protection plays a crucial role in the maintenance of spermatozoan membrane integrity and their fertilising ability. Boar spermatozoa are sensitive to peroxidation damage due to their high content of unsaturated fatty acids in the phospholipids of their plasma membranes (Cerolini *et al.* 2000). Therefore, antioxidant protection is a vital element in maintaining spermatozoa membrane integrity, motility and fertilizing ability (Surai and Fisinin, 2015). Spermatozoa are efficient producers of reactive oxygen species (ROS), which are involved in the decreased motility and viability of spermatozoa (Am-in *et al*., 2010).

Reproduction of sows and boars is sensitive to selenium deficiency, and meeting their requirements is an important challenge for pig nutritionists (Audet *et al.,* 2009). In fact, in many countries, there are legal limits as to how much selenium can be included into the diet and this restricts flexibility in terms of addressing the selenium needs of breeding pigs (Surai *et al.,* 2015). It is necessary to take into account that only an optimal selenium status of animals is

associated with the best antioxidant protection and could have positive effects on boar semen production quality. However, in many cases, selenium levels are not determined and therefore, it is difficult to judge if the basic diets were deficient in selenium. The most important anti-oxidants include zinc, vitamins C and E (Surai *et al.*, 2001). Selenium and zinc are a part of anti-oxidant enzymes-glutathione peroxidase and superoxide dismutase, respectively (Klusoňová *et al*., 2015). In boars, there is higher production of free oxygenradicals able to damage cells, including boar spermatozoa.

In humans and other animal species the blood levels of molecules related to lipid, glucose, non-esterified fatty acids, triglyceride, glucose and alanine aminotransferase (ALT), reflect both nutritional and disease status. Consumer demand for lean meat which is on the increase (Peres *et al*., 2014), therefore, challenge in pig industries is to improve the nutritional value, quality, and shelf life of pork. However, selenium supplementation might improve these growth and reproduction traits (Oliveira *et al.*, 2014) and, in addition, maintain a healthy immune system of both the pigs and consumers.

The interaction between genotype, selenium and zinc should be understood to benefit pig producers, researchers, consumers and processing companies. Consumers will also be able to get cheap quality pork that is socially acceptable. Determination of the interaction between selenium and zinc supplementation in Kolbroek boar diets is highly beneficial in improving reproductive efficiency of breeding sires, maintain genotype diversity and, consequently, improve livehoods of resource-poor farmers with a concomitant economic benefits to the pig industry. Researchers will also able to develop their understanding of mineral requirements to optimise fertility in boars and to develop appropriate advisory materials for farmers and feed

compounders. Improvement of pig performance is likely to enhance profitability of pig enterprises through appropriate mineral supplementation.

1.3 Objectives

The broad objective of the study was to assess the interaction of dietary selenium and zinc supplementation on growth performance and semen quality and carcass, traits of Kolbroek and LW × LR boars. The specific objectives were to:

1. determine the interaction of selenium and zinc supplementation ongrowth performance of LW × LR crossbred and Kolbroek pigs.
2. examine the interaction of Se and Zn supplementation on sexual behaviour, ejaculation volume, testosterone concentrations in LW × LR crossbred and Kolbroek pigs.
3. determine the interaction of selenium and zinc supplementation on carcass characteristics of LW × LR crossbred and Kolbroek pigs
4. assess the interaction of dietary selenium and zinc supplementation on epidydimal semen production quality, lipid peroxidation and seminal plasma components of LW × LR crossbred and Kolbroek pigs; and
5. determine the interaction of selenium and zincsupplementation on testicular morphology, spermatogenesis and histology of LW × LR crossbred and Kolbroek pigs

1.4 Hypotheses

1. Selenium and zinc supplementation have no effects on growth performance of growing LW × LR crossbred and Kolbroek pigs, and there is no interaction between the two.

2. Selenium and zinc supplementation have no effects on ejaculation volume, testosterone, sexual behaviour and luteinising hormone concentrations of growing LW × LR crossbred and Kolbroek pigs, and there is no interaction between the two.
3. Selenium and zinc supplementation have no effects on carcass characteristics of growing LW × LR crossbred and Kolbroek pigs, and there is no interaction between the two.
4. Selenium and zinc supplementation have no effect on on epidydimal semen production quality, lipid peroxidation and seminal plasma components of LW × LR crossbred and Kolbroek pigs and there is no interaction between the two.
5. Selenium and zinc supplementation have no effects on testicular morphology, spermatogenesis and histology of growing LW × LR crossbred and Kolbroek pigs, and there is no interaction between the two.

1.5 References

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Chapter 2

Literature review

2.1 Introduction

The absolute requirements for one nutrient can be influenced by the amounts or proportions of other nutrients in the diet (Campbell *et al.*, 2000). Selenium and zinc are important for normal reproductive function in males. They exert beneficial effects on the reproductive function of both males and females and, therefore, have drawn great interest among researchers in the past years aiming to acquire a better understanding of their function in pig diets. Zinc is commonly added in pig diets because a large number of natural feedstuffs are marginally zinc deficient. Selenium is an essential mineral that is routinely added in all pig diets via trace-mineral premixes. The aim of the review is to give an overview on the physiological roles of selenium x zinc interaction that commandeer the best fertility in boars. The review focuses on the importance and measures of boar fertility, factors affecting boar fertility and role of selenium and zinc on fertility.

2.2 Importance of boar fertility

A fertile boar is the one that expreses the highest level of sexual behaviour and characterized by the highest ejaculate volume, the best quality of ejaculate and the high number of insemination doses from one ejaculate. Understanding boar fertility is important for pig producers because the impact of each male on herd performance is high, particularly when artificial insemination is used (Juonala *et al.*, 1998). Recently, the identification of genetic markers for boar fertility has been found to be highly valuable to improve boar fertility and to

identify more efficient semen producers prior to boar selection (Tremoen, 2018). Boar fertility is of great importance for overall pig reproduction efficiency and the profitability of pig producers (Tremoen, 2018).

The most valid assessment of boar fertility is to obtain viable pregnancies and viable offspring following insemination (Gadea, 2005). Since fertility has low heritability, it is likely to be improved through crossbreeding and genomic selection (Lekule *et al.*, 1990). Boars associated with a high fertility rate and large litters consistently produce inseminations that contain sufficient numbers of spermatozoa (Koketsu *et al*., 1999). Although boar fertility plays an important role in the efficiency and productivity of the system, semen traits are usually absent from selection decisions (Rothschild, 1996; Smital *et al*., 2005; Ruiz-Sanchez *et al.*, 2006). Genomic selection could be an alternative to improve boar fertility traits earlier in life. (Broekhuijse *et al.*, 2012).

Masenya *et al.* (2011) reported that there is lack of accurate methods of predicting the fertility of Kolbroek boars to determine their reproductive potential. Therefore, the need to measure cellular features associated with fertility and examine their relationship between boar fertility and spermatozoa motility with litter weight and size is important. Given the importance of boar fertility and the need to include it in selection criteria, it is vital that measures of boar fertility are clearly understood.

2.3 Measures of boar fertility

The commonly used to measures of boar fertility are litter size, libido, scrotum size in boars, ejaculation volume and semen quality.

2.3.1 Litter size

If boars are over-worked, a reduction in litter size is likely (Peadar *et al.,* 2007). The lower fertilization and farrowing rates and litter size can occur as a result of semen backflow or leakage (Steverink *et al.,* 1998). Selection of boars from a prolific dam line, gradually increase litter size over time because litter size and its component traits respond to selection (Johnson *et al.,* 1999). These authors showed that selected boars showed lesser zona binding ability and a small average litter size, but greater farrowing rate (80 %). Boe-Hansen *et al.* (2007) reported that total number of piglets born for Hampshire, Landrace and Danish Large White boars was 0.5, 0.7 and 0.9 piglets smaller per litter, respectively when daily feed intake values were above 2.1 % as opposed to below this value. Umesiobi (2010) reported that the semen collected at 96-h gave higher non-return rate (93.5 ± 2.9 versus 76.8 ± 5.2 %), farrowing rate (85.5 ± 14.3 versus 56.8 ± 9.1%,), litter size (12 ± 0.03 versus 8 ± 0.02) and live piglets were 30 % higher compared with those from sows inseminated with semen collected at 24-h intervals. Umesiobi (2010) reported that boar semen with the highest semen quality and quantity, and the largest number of piglets per litter with the greatest proportion piglets farrowed alive. Our data suggest that litter size directly increases with the proportion of capacitated spermatozoa (Oh *et al.,* 2010).

Correlation estimates for Large White indicated that increased litter size in the first litter was genetically associated with a slight increase in semen volume (0.18 to 0.21), a decrease in spermatozoa concentration (−0.30) and motility (−0.39 to −0.49), and an increase in the percentage of abnormal spermatozoa (0.55 to 0.63) (Wolf, 2010).

2.3.2 Libido

Libido or sexual desire, showed by reaction time, is an important aspect of boar reproductive function (Umesiobi and Iloeje, 1999). Madsen *et al.* (1992) highlighted that libido is the primary determinant of boar fertility. It may be impaired by mismanagement of young boars during service (Hafez and Hafez, 2000). Boars with high testosterone levels also exhibit high levels of libido (Flowers, 2008). More distinct sexual drive is probably a consequence of a higher level of testosterone in blood, and Williams (2009) indicated a close relationship between the level of testosterone and sexual behaviour and libido. Low levels of sexual behaviour result from either low sexual motivation or poor mating competency (Hemsworth and Tilbrook, 2007). Evaluation of libido is performed on the basis of duration of preparation time for a jump onto sows in oestrus and duration of erection. When testing libido, dummy sows are often used to determine the libido levels of boars. The duration of ejaculation or total time elapsed from the entrance into the room for spermatozoa collection to the end of ejaculation is recorded (Okere *et al.,* 2005; Szostak and Sarzyńska, 2011; Oberlender *et al.,* 2012). Boars with high levels of libido are likely to yield higher reproductive efficiency of the sows.

2.3.3 Scrotum size

The role of the scrotum is to regulate testes temperature to be lower than body temperature (Knox, 2003). Scrotum circumference before puberty determines the rate of spermatozoa production in sexually mature boars (Gnessi *et al.,* 1997). The cremater muscle found in the spermatic cord and contracts or body in cold weather or let them hang further away in hot weather (Knox, 2003). Thus, there is an increase in the tubular diameter and also the scrotal circumference (Assis Neto *et al*., 2003). Testicular size is a good predictor of spermatozoa production (Rathje *et al.,* 1995; Huang and Johnson, 1996). It has a moderate to high

heritability (Bidanel, 2011), indicating the possibility of efficient selection. The weight of testes is correlated with daily spermatozoa production and total spermatozoa reserves (Lubritz *et al.*, 1991; Rathje *et al.*, 1995). Selection for increased size of testes at 150 d of age in boars was practised for 10 generations (Johnson *et al.*, 1994) and boars from different lines had testes weight that increased at the same rate (Rathje *et al.*, 1995). The large differences in weight of testes between the selected and control lines explain most of the advantages in total daily spermatozoa production for boars (Harder *et al*., 1995). Weight of testes at a constant age may, therefore, be a useful indicator trait to select for increased reproductive efficiency of boars (Johnson *et al.* (1994).

2.3.4 Semen volume

The most important quantitative and qualitative traits of ejaculate are volume of ejaculate and concentration of spermatozoa and number of doses produced per ejaculate (Savić *et al.*, 2017). Petrović *et al*. (1994) reported an unfavourable effect of isolation of boar on the decrease of ejaculate volume. An acceptable level of volume of ejaculate occurred after a 3-day sexual pause, spermatozoa reserves replenished after five to seven days, while full recovery takes about 10 days (Smital, 2009). Savić *et al.* (2015) showed a significant effect of interval between two jumps on the volume of ejaculate, total number of spermatozoa and total number of doses per ejaculate. The volume of ejaculate, the effect of interval between the two semen collections is considerably weaker and increases when the interval is prolonged from two to seven days (Savić *et al.*, 2017). At higher collection frequency, the production of spermatozoa per unit time was increased but the number of spermatozoa per ejaculate was decreased (Frangeî *et al.*, 2005). High collection frequencies also decrease spermatoza motility, concentration, total spermatozoa and increased percent abnormal spermatozoa (Strzezek *et al.*, 1995; Broekhuijse *et al*., 2012). No information on semen volume is available in Kolbroek pigs.

2.3.5 Semen quality

Boars that expressed the highest level of sexual behavior were characterized by the higher percentage of spermatozoa with progressive motility and the small percentage of defective spermatozoa (Szostak *et al.,* 2015). Flower (2015) reported that the boars had shorter reaction times (2.5 versus 3.0 s), longer collection times (372.3 versus 319.1 s), greater collection volumes (230.4 versus 194.1 mL) and more total spermpermatoza ejaculate (68.1 billion versus 63.1 billion). Secondly, ejaculating boars at 96-h intervals enhanced semen quality and quantity leading to significant improvements in the fertility and litter size of artificially inseminated sows (Umesiobi, 2010). Boars associated with a high fertility rate and large litters consistently produce inseminations that contain sufficient numbers of spermatozoa capable of completing all of these tasks (Koketsu *et al.,* 1999; Willenburg *et al.,* 2003). The negative predictive value is determined as the percentage of boars that test negative but have a litter size of ≥12 or <12 (Kwon *et al*., 2013). The average litter size significantly increased from 11.1 to 11.98 by using boar semen (Kwon *et al*., 2017). The number of live-born piglets (10.82) was larger following using a 50 mL volume dose with a 2×10^9 spermatozoa count as compared with the same artificial insemination (AI) dose volume and spermatozoa count (9.85) (APIĆ *et al.,* 2015).

Capacitation and the subsequent acrosome reaction are essential for the *in vitro* penetration assay (Oh *et al.* 2010). Therefore, the hyperactivation, curvilinear velocity, the mean amplitude of head lateral displacement, linearity, wobble, acrosome-reacted spermatozoa, and capacitated spermatozoa increased after the induction of capacitation regardless of the litter size. Non-capacitated spermatozoa decreased after capacitation regardless of litter size (Kwon *et al*., 2015). The percentage of spermatozoa with normal morphology also explained a large part of the variance in litter size, indicating that morphological characteristics are a useful measure of

semen quality (Xu *et al.,* 1998). Wolf (2010) reported that the variation in size of the first litter was not associated with variation in semen volume, spermatozoa concentration or motility, but was associated with decreased percentage of abnormal spermatozoa. Second and subsequent litter size were significantly correlated only with percentage of abnormal spermatozoa (Wolf. 2010). Feitsma (2009) reported that a 10 % increase in abnormal spermatozoa decreased litter size by 0.1 piglets. Foxcroft *et al.* (2008) reported that the hypo-osmotic swelling test resistance of the boar spermatozoa was correlated with fertility. Watery semen can be a sign of low spermatozoa count, indicating possible fertility problems. Ejaculating thin, clear semen may also be a temporary condition with no serious health concerns. There is no information available in semen quality in Kolbroek pigs.

These measures of fertility have been explored extensively in improved genotyes, yet information on Kolbroek and other local genotypes is poorly understood. Factors affecting boar fertility in Kolbroek boars also needs to be understood.

2.4 Factors affecting boar fertility

This ability is limited by testicular capacity, libido and physical soundness (feet, legs, back). A lot of studies indicate that the reproductive fitness of boars depends on the the heritability (Oh *et al.,* 2003), testicular size (Clark *et al.,* 2003), nutrition (Khan *et al*., 2005), age of the boar (Jankeviciute and Zilinskas, 2002), intensity of sexual exploitation (Frangez *et al.,* 2005), photoperiod (Sancho *et al*., 2004), outdoor temperatures, rhythm of semen collection, accuracy of semen processing and social environment (Hemsworth and Tilbrook 2007; Kunavongkrit *et al.,* 2005).

2.4.1 Effects of nutrition on boar fertility

The reproductive performance of boars can best be described by evaluating three characteristics: i) libido; ii) spermatozoa production, and iii) spermatozoa viability and fertilisation capacity. Nutrition is critical to a boar's sexual and reproductive development and performance (Close and Cole, 2003). The nutrient requirements for reproduction are low to meet the boars metabolic need (Mahan, 1990) but under-nutrition results in adverse effects on the reproductive capacity of boars (Cheah and Yang, 2011). Therefore, successful reproduction requires adequate provision of proeins, minereals, vitamins and fats (Cheah and Yang, 2011). The energy requirements for mating activity and spermatozoa production are small.

2.4.1.1 Protein content of diets

Low protein in the diet reduced boar semen volume and libido after seven weeks (Louis *et al.,* 1994). Boars fed on diets with a protein level of 130 g/kg, but high level of threonine: tryptophan: arginine ratio of the protein content showed enhanced boar semen quality (Ren *et al*., 2015). An increase in protein in the diet did not increase levels of libido of boars (Rooke *et al*., 2001). There is, however, influence of dietary protein levels on libido of Large White boars (Kemp, 1991). In addition, protein is important for spermatozoa quality (Wilson *et al.,* 2004) (Machebe *et al.,* 2014). Boars with low protein intakes had reduced libido and semen volume (Louis *et al.*, 1994). Low protein intakes resulted in decreased spermatozoa production in rats (Vawda and Mandlwana, 1990) and bulls (Rekwot *et al.,* 1988). Louis *et al*. (1994) reported that boars fed on higher crude protein diet (160 g/kg CP) recorded 26 % more semen volume than those fed on a diet containing 70 g/kg. Brown (1994) and Machebe *et al*. (2014) reported that the increasing dietary protein intake increased libido and semen volume. Boars with a low protein intake took longer to start ejaculating and remained on the semen-collection dummy for a shorter time than boars on the control treatment (Louis *et al.,* 1994). Kemp (1991)

reported that the pigs fed on low protein levels had low numbers of spermatozoa ejaculated and increases in number of spermatozoa was found at higher protein levels. Protein intake, therefore, ensures a good libido and maintain the numbers and quality of spermatozoa produced by AI boars (Kemp, 1991).

Dong *et al.* (2013) reported that low protein diets, when in combination with low energy intake, reduces boar interest in mounting adummy sow and ejaculation events. Similar results in the way of increased volume and spermatozoa concentration when animals were fed on higher crude protein diet have been obtained in boars (Louis *et al.,* 1994), rat (Vawda and Mandlwana, 1990) and bulls (Rekwot *et al.,* 1998). In addition, there is no information on how protein supplementation influence the spermatozoa production in Kolbroek pigs. It is therefore, pertinent that future research should further explore these areas.

2.4.1.3 Calcium

Calcium is the most deficient mineral in diets formulated with cereal grains and oilseed meals (Campbell *et al.,* 2000). As calcium level increases, spermatozoa motility during capacitation in boar spermatozoa also improves (Zhou *et al.,* 1990; Dube *et al.,* 2003). Rahman (2014) reported decreased spermatozoa motility with increased calcium deficiency. Parodi (2014) reported that calcium supplementation also increases the reactive oxygen species. Chauhan *et al.* (1998) reported that increasing the level of calcium in buffaloes led to increased motility of viable spermatozoa. Calcium supplementation has been shown to increase spermatozoa motility by 60 % (Shirakawa and Miyazaki, 1999). In addition, there is no information on how does calcium influence the spermatozoa production in Kolbroek pigs.

2.4.1.4 Phosphorus

Phosphorus is an essential element, being the second most abundant mineral in the animal's body after calcium. Tardif *et al.* (1999) reported that phosphorous mediates the onset of capacitation and spermatozoa production in pigs. In human spermatozoa, phosphorous increases capacitation, and increases motility of spermatozoa velocity (Leclerc *et al*., 1996). The influence of phosphorus on spermatozoa production in Kolbroek pigs is poorly understood.

2.4.1.5 Magnesium

Magnesium is an essential dietary mineral. The requirement for magnesium for pigs has not been clearly defined but has been suggested to be approximately 0.04 mg/kg (Van Heugten, 2009). Supplementation of boars with magnesium improved conception rate of sows and service interval by nine days (Zang *et al.,* 2014). Magnesium deficiency reduces reproductive efficiency and leads to loss of appetite (Sathish, 2003). No research has reported the influence of magnesium on spermatozoa production in Kolbroek boars (Zang *et al*., 2014).

2.4.1.6 Copper

Copper is an essential trace nutrient playing important roles in general health and fertility of boars (Tvrda *et al*., 2015). Yuyan *et al*. (2008) indicated that increased copper concentration decreased the percentage of progressively motile spermatozoa of men. Gamcik *et al.* (1990) reported that the toxic effects of copper on seminal plasma are manifested in the decrease in the percentage of motile spermatozoa and in the decrease of malformed spermatozoa in rams. Aghaei *et al.* (2010) reported that increasing copper concentrations in rooster seminal plasma led to an increase in the progressive motility percentage of spermatozoa. A low concentration of copper has been reported to cause a decrease in spermatozoa motility (Slivkova *et al*., 2009). Tabassomi *et al.* (2013) reported that increasing of copper levels increased the seminal antioxidant capacity and reduction of oxidative stress status in water buffaloes (*Bubalus*

bubalis). Yuyan *et al.* (2008) and Bombardelli *et al.* (2016) reported that copper supplementation significantly decreased the percentage of progressively motile spermatozoa. There is no information on how copper influences the spermatozoa production in Kolbroek pigs.

2.4.1.7 Zinc

Zinc plays several roles in the boar reproductive system. It contributes to the ribonuclease activity that is highly active during mitosis of spermatozoa and meiosis of spermatocytes (Kaur and Bansal, 2005). Zinc supplementation indicated no increase in motility scores, number of doses rejected, and morphological examination scores (Althouse *et al.*, 2000). Zinc increases male fertility by regulation of the expression of testis during the differentiation process and spermatogenesis (Ghasemzadeh-Hasankolai *et al.*, 2012; Fathi and Farhzadi, 2015). Zinc supplemetation also improves spermatozoa density, progressive motility and improved conception and pregnancy outcome in men (Khan *et al.*, 2011; Fallah *et al.* 2018). Arangasamy *et al.* (2018) reported that zinc supplementation increases the semen volume, spermatozoa concentration and improved the spermatozoa membrane integrity in bucks. Zinc supplementation also improved sexual behaviour (libido) in a male rats (Dissanayake *et al.*, 2009). Krishnaiah *et al.* (2019) reported that zinc supplementation decreased total mount attempts, flehmen reactions and the number of mounts without ejaculation in bucks.

Zhao *et al.* (2016) reported that zinc supplementation increased semen volume, spermatozoa motility and percentage of normal spermatozoa morphology of infertile males, suggesting that zinc supplementation might increase male reproductive function. Telisman *et al.* (2000) reported that a zinc increase was associated with the increase in counts of total and viable spermatozoa and motile and viable spermatozoa in men. Kumar *et al*. (2006) reported that zinc

supplementation increased semen volume, spermatozoa concentration, live spermatozoa and spermatozoa motility in bulls. Zinc supplementation also deceresed spermatozoa density, viability, and penetrating capacity (Guanglin and Zhiyu, 2000; Shiming *et al.,* 2002). Kumar *et al.* (2006) reported significantly higher values of ejaculate volume with increased of zinc supplemented in bulls. No information on how zinc supplementation influences spermatozoa production in Kolbroek pigs is available.

2.4.1.8 Selenium

Marin-Guzman *et al*. (2000) showed that selenium is involved in a regulation of boar spermatozoa maturation in the epididymis. Selenium has received much attention for its antioxidant properties as a structural component of glutathione peroxidase (GPx). The enzyme is present in boar spermatozoa which protects cellular and subcellular membranes against peroxidation (Jelezarsky *et al.,* 2008). In boars, supplementation of a diet containing 0.06 mg/kg selenium with 0.5 mg/kg selenium from weaning to nine months of age resulted in higher spermatozoa motility, increased spermatozoa concentrations and fertilization rates (Marin-Guzman *et al.,* 1997). Spermatozoa motility with cytoplasmic droplets increased when boars were fed on a low-selenium diet (Marin-Guzman *et al*., 2000). Supplementing boars with 0.6 mg/kg selenium improved boar ejaculate characteristics (Horky *et al.,* 2012). Marin-Guzman *et al.* (2000) reported that boars supplemented with selenium at the age of 18 months had increased numbers of spermatozoa reserves. Lopez *et al.* (2010) indicated that selenium in the diet of boars increased spermatozoa concentration but reduced some motility parameters and resistance to oxidative stress. Kolodziej and Jacyno (2005) indicated that 0.5 mg/kg of selenium increased spermatozoa concentration and total numbers of spermatozoa quality compared to boars fed on diets containing 0.2 mg/kg selenium.

Surai and and Fisinin (2015) reported that as the dietary selenium in diet increased, there was an increased in semen volume, concentration, total spermatozoa, spermatozoa motility, progressive motility, morphology, lipid peroxidation. The increased selenium reduced reation of oxidative stress (ROS) formation and enhanced the preservation of the integrity of the spermatozoa membranes (Hansen and Deguchi, 1996; Ursini *et al*., 1999). Maiorino *et al.* (1999) reported that that males consuming diets low in selenium produced spermatozoa with low motility and increased abnormalities. Lovercamp *et al.* (2013) reported that increased levels of dietary selenium supplementation increased semen volume, concentration and total spermatozoa and spermatozoa progressive motility in boars. Boars that were supplemented with selenium showed increased progressive motility of the spermatozoa, resistance to hypo-osmotic shock and therm altests (Petrujkic *et al.*, 2014). Li-guang *et al.* (2010) reported that spermatozoa production increased significantly when selenium supplementation levels of 0.3 mg/kg were used, in comparison with control received 0.06 mg/kg in Boer goats. Słowińska *et al.* (2011) reported, however, that selenium supplementation decreased the quality of semen through an increase activity of seminal plasma and a decrease in some spermatozoa motility parameters in turkey. Information on the influence of zinc and selenium supplementation influence spermatozoa production in Kolbroek pigs is scarce.

2.4.2 Age of boar

The dependence of spermatozoa concentration on age started with a short increase until 12 months followed by a long-term moderate decrease until three years of age and a relative stabilization thereafter (Wolf and Smita, 2009). At three months of age, there is a second period of germ cell division and rapid increase in the testes to body weight ratio. Jankeviciute and Zilinskas (2002) reported that, at four months of age, spermatozoa first appear in the seminiferous tubules and erection can be accomplished in the semen ejaculate. Over the next 6

to 18 months, the testes increase in size and both semen concentration and ejaculate volumes continue to increase. Wolf and Smita (2009) reported that semen volume and concentration of spermatozoa increased until an age of about two years by approximately 100 ml and remained more or less constant thereafter in boars. Boars reach puberty between five and six months, waves of spermatozoa are released from Sertoli cells every three to four days and acquire fertilizational competence after an additional five to seven weeks of maturation (Garner and Hafez, 1993). The testicles of boars born with high birthweight were 16 % heavier and contained 53 % more spermatozoa compared with their low birthweight counterparts at eight months of age (Almeida *et al.,* 2013; Dysart, 2015).

Bonet *et al.* (1991) reported that the prolonged and very frequent collection of ejaculates (every two days over 12 months) leads to a decrease in the percentage of progressively motile spermatozoa in the ejaculates. Šerniene *et al.* (2002) reported an increase in the percentage of abnormal spermatozoa with age. Clark *et al.* (2003) found a dramatic increase in the average total number of spermatozoa between boars of eight to 10 months and up to 14 months of age followed by constancy in this trait after 14 months of age. Most boars reach puberty (ability and willingness to ejaculates fertile spermatozoa) between 5 and 8 months of age (Rutten *et al*., 2000). Spermatozoa may be found in the testes much earlier (110 to 125 days of age) than this, but there is some delay before the spermatozoa production are able to fertilize ovary and the boar develops the coordinated pattern of sexual behaviour necessary for successful copulation (Levis *et al*., 1997). Information on Kolbroek pigs is scarce.

2.4.3 Boar selection

Selecting boars with the best semen quality at an early age is imperative to reduce the costs of raising animals that will not be used for semen production. The most important part in

reproductive management is the control of boar fertility (Savić *et al*., 2017). The boar fertility traits are represented by *in vitro* (spermatozoa traits) and *in vivo* (reproductive efficiency and litter size) fertility. In pig populations where selection is practised, the boars express the highest level of sexual behaviour are are characterized by the greatest number of ejaculate, the best quality of ejaculate (the higher percentage of spermatozoa with progressive motility, the small percentage of defective spermatozoa) and the high number of insemination doses from one ejaculate (Szostak *et al.*, 2015). In pig populations where continuous selection is conducted, there boars of good production performances are used in the best way possible to produce more doses per ejaculate of optimal fertilising capacity as possible (Flowers, 2002; Savić *et al.*, 2013). Boars are being selected mostly on the traits having a primary economic importance such as the rate of bodyweight gain or the age at certain body mass, bacon thickness and productivity of their daughters (Robinson and Buhr, 2005). There is no information on Kolbroek pigs.

2.4.4 Ambient temperature and humidity

Pigs are inefficient at using sweat to cool their bodies during high ambient temperatures (Einarsson *et al*. 2008). Marchev *et al.* (2003) reported that semen collected in autumn and winter had significantly higher semen volume and spermatozoa concentration. Boars suffer from acute and persistent exposure to elevated ambient temperatures. Boars that have been exposed to a controlled hot-room environment, direct sunlight or ambient temperatures ranging from 30 to 40°C for between 3 and 90 days exhibited a significant decrease in spermatozoa motility, normal morphology and spermatozoa concentration (Patil *et al.*, 2013). Exposure to heat i.e. testicular insulation, scrotal heating, dipping of testes into hot water and heated incubation decreased spermatozoa, fragmentation of spermatozoa DNA in pigs (Fernandes *et al*., 2008).

Low fertility and low spermatozoa concentration and decreased ejaculate volume have been found in boars during or shortly after the warm summer period (Waberski *et al.*, 1994). Boars exposed to ambient temperatures of more than 29°C had lower spermatozoa output and poorer spermatozoa motility and increased morphological abnormalities (Parrish *et al.*, 2016). Sonderman and Luebbe (2008) and Flowers (2008) reported that thermal stress negatively decreased spermatozoa quality and ejaculation volume in boars. A high ambient temperature and humidity reduces semen production and semen quality. High temperatures also reduce boar libido which reduces his ability to detect oestrus. Temperatures between 15 to 20°C can be tolerated by semen production. Temperatures outside this range are detrimental to semen and reduce the number of viable spermatozoa and the shelf-life of the semen

The influence of temperature and boar spermatozoa motility, viability and acrosome integrity of spermatozoa is shown in Figure 2.1 (Rakmali *et al.*, 2015). Flower (2015) indicated that the motility spermatozoa had affected by ambient temperatures. Kunavongkrit *et al.* (2005) reported that a large temperature range (difference between maximum and minimum temperature) decreased spermatozoa motility in boars. Several studies reported that, in the summer-autumn period, there was low semen quality traits (Smital, 2009; Petrocelli *et al.*, 2015). Pérez-Llano *et al.* (2010) reported that increased temperature decrease spermatozoa viability, spermatozoa DNA integrity, and the offspring sex ratio in boars. Boars showed increased of semen volume with the increased temperatures, but spermatozoa motility decreased (Corcuera *et al.*, 2002). Wolf and Smital (2009) reported that the temperature control within boar studs may help with the increased semen quality throughout the year. Feitsma and Grooten (1993) reported that the ambient temperatures, heat stress and/or hot weather reduced spermatozoa motility and spermatozoa morphology in boars. Petrocelli *et al.* (2015), however,

argued that since the temperature in the boar housing did not reach values that could cause heat stress and decreased spermatogenesis, it could be assumed that the main effect of the season is photoperiod. There is no such information in Kolbroek pigs.

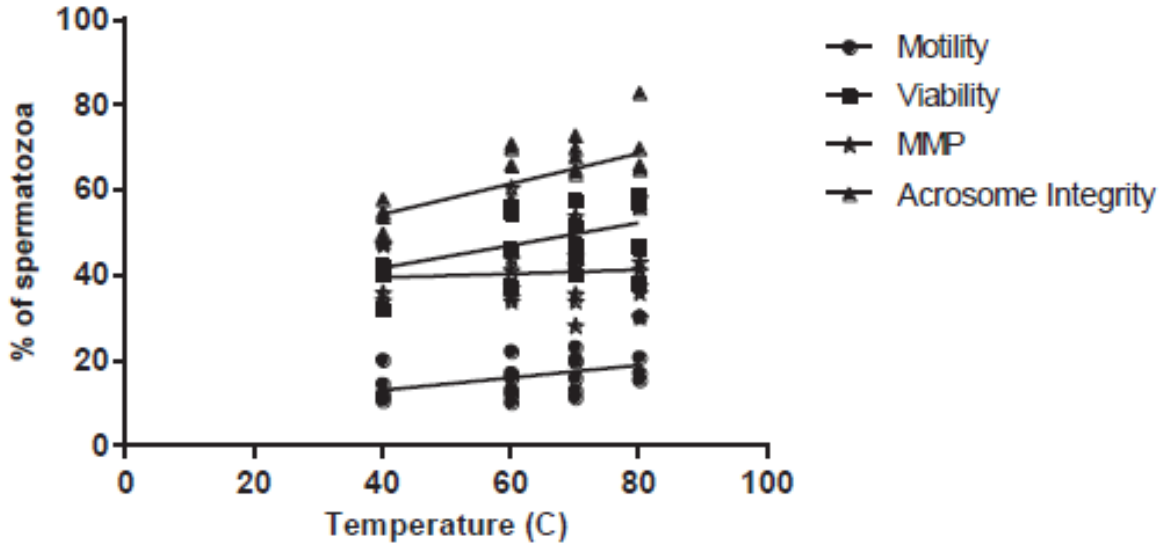

Figure 2.1: The influence of tempature and boar spermatozoa motility, viability and acrosome integrity of spermatozoa (Rakmali *et al*., 2015).

2.4.5 Photoperiod

The effect of increasing and decreasing photoperiod on semen volume, including months and breeds is shown in Figure 2.2. Petrocelli *et al.* (2015) reported that autumn and the decreasing photoperiod had a negative impact on most of the semen characteristics, except for volume. Knecht *et al*. (2013) reported that semen volume was higher during the decreasing period in photoperiod. Sancho *et al.* (2006) reported that boars at 24 h of artificial light or 24 h of complete darkness for three months reduced semen volume and spermatozoa concentration. Petrocelli *et al.* (2015) reported that decreasing daily photoperiod and high ambient temperature seemed to play a role of increasing semen quality traits. Marchev *et al.* (2003) and

Wolf and Smital (2009) reported that with decreasing photoperiod, semen volume and concentration was increased. Sancho *et al.* (2004) reported that a short light period was associated with a decline in ejaculate volume, spermatozoa concentration, total number of spermatozoa in an ejaculate in Landrace boars.

The effect of increasing and decreasing photoperiod on spermatozoa concentration, including months and breeds is shown in Figure 2.3. Ciereszko *et al.* (2000) reported that spermatozoa concentration decreased by decreasing photoperiod. Sancho *et al*. (2004) reported that spermatozoa production was lower in boars under a decreasing photoperiod than boars under an increasing photoperiod. Smital (2009) and Sancho *et al.* (1998) reported that a decreasing photoperiod induced a concomitant decrease related to the boar-spermatozoa formation, such as spermatozoa concentration and the percentage of proximal cytoplasmic droplets. Sancho *et al.* (2004) indicated that semen quality of boars exposed to a decreasing photoperiod was reduced and Kozdrowski and Dubiel (2004) showed that spermatozoa motility was lowest in summer.

The effect of increasing and decreasing photoperiod on the total number of motile spermatozoa, including months and breeds is shown in Figure 2.2. Few studies on the effects of photoperiod on semen parameters are available (Rivera *et al.*, 2005; Sancho *et al*., 2004). Love *et al.* (1993) showed a decrease in spermatogenesis in peripubertal boars (Andersson *et al*., 1998). Yeste *et al*. (2010) indicated that even use of L-carnitine added during the increasing photoperiod did not improve the performance of semen in Large White and Duroc boars. The influence of photoperiod in Kolbroek pigs is largely unknown.

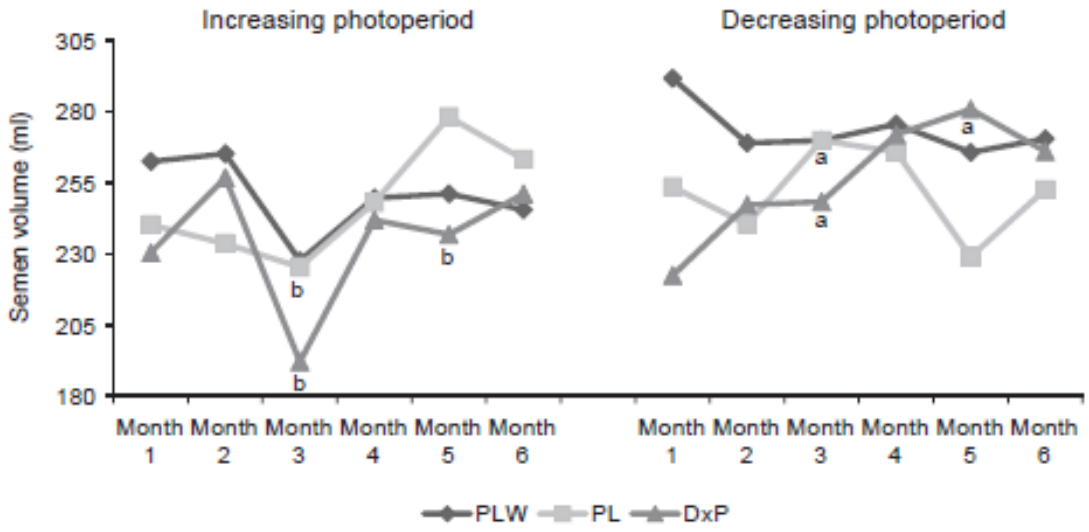

Figure 2.2: Effect of increasing and decreasing photoperiod on semen volume, including months and breeds

[a, b] Significant differences between the same breeds of boars (Knecht *et al.*, 2013).

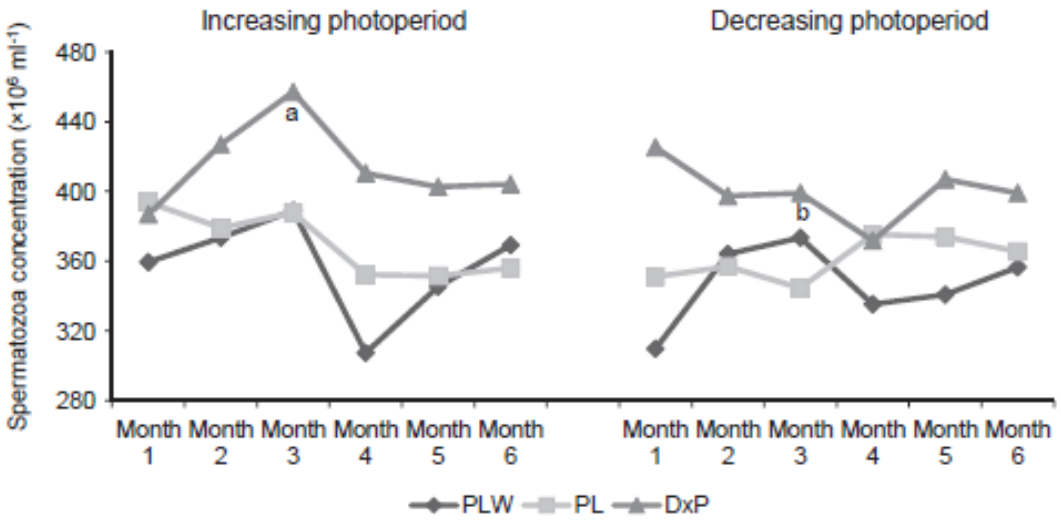

Figure 2.3: Effect of increasing and decreasing photoperiod on spermatozoa concentration, including months and breeds

[a, b] Significant differences between the same breeds of boars (Knecht *et al.*, 2013).

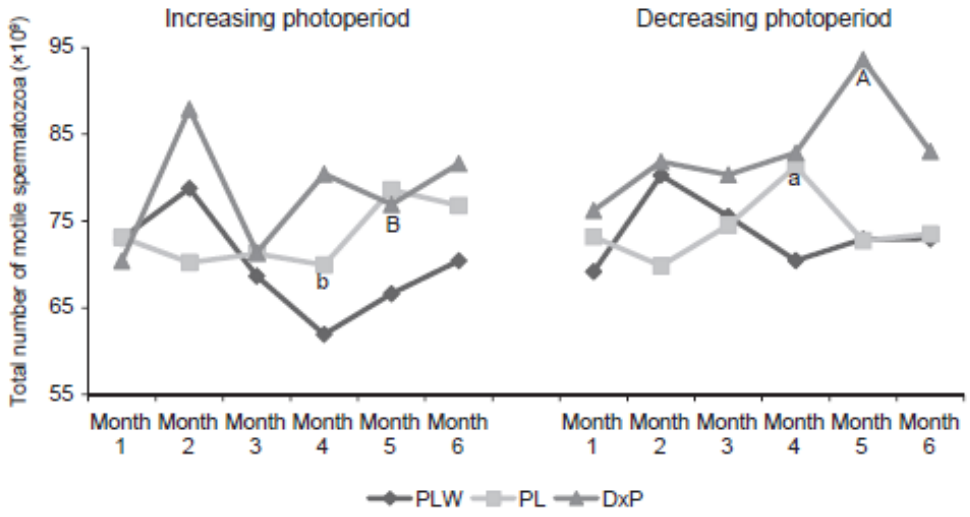

Figure 2.4: The effect of increasing and decreasing photoperiod on the total number of motile spermatozoa, including months and breeds

[a, b] Significant differences between the same breeds of boars (Knecht *et al.*, 2013).

2.4.7 Season

In most pig units, a decrease in semen volume and spermatozoa production is observed in spring (Feitsma and Grooten, 1993). Kunavongkrit and Prateep (1995) reported that the semen volume and spermatozoa concentration in Duroc boars was lowest during the hot season. Kozdrowski and Dubiel (2004) reported that a reduction in both ejaculate volume and total spermatozoa production was found during the hot season in both the conventional open and the evaporative cooling housing systems. There was a decrease in ejaculate volume between January and May and a decrease in daily spermatozoa production between January and March (winter and early spring) (Colenbrander and Kemp, 1990; Colenbrander *et al*., 1993).

The highest semen volume was reported in autumn and the lowest in spring, as also confirmed by other researchers (Frydrychová *et al.*, 2007; Table 2.1). Knecht *et al.* (2014) reported that the highest spermatozoa concentration was observed in autumn and winter, and the lowest in the higher temperature period of spring and summer. Xue *et al*. (1994) reported that the lowest semen production of commercial pigs was found in summer. Brito *et al.* (2002) reported that a decrease in percentage of morphologically-altered spermatozoa in summer. Flowers (1997) reported that year on year, temperature is the same pattern of an increase in the number of abnormal spermatozoa morphologies or poor-motility spermatozoa. Banaszewska *et al*. (2007) reported that the decrease in ambient temperature after summer increases the concentration of spermatozoa in autumn. Flowers (2002) reported a much lower number of spermatozoa in ejaculate in the summer months compared with the remaining months. Wolf and Smital (2009) reported no seasonal differences were found, re-enforcing recent work which demonstrated that motility spermatozoa remained relatively constant and seasonal differences in percentage of abnormalities are negligible.

Effect of season on spermatozoa acrosin activity and number of spermatozoa in ejaculate and spermatozoa concentration of of boar semen is shown in Figure 2.5. Wysokińska *et al.* (2009) reported that ejaculates collected in November and December had 20 mL greater volume than ejaculates collected in spring (March, April and May). The causes of seasonal fluctuations in semen quality are not fully understood, but likely are mediated by hormonal mechanisms controlled by photoperiod. Xue *et al.* (1994) reported that the seasonal mechanisms likely still influence semen characteristics of boars, especially semen volume and number of spermatozoa in the ejaculate were in spring. For this reason, seasonal changes of semen quality characteristics, such as membrane integrity of boar spermatozoa, decreased in summer (Ciereszko *et al.,* 2000). De las Heras *et al.* (1996) reported that a significant decreased of spermatozoa morphology in October and February.

High season temperatures could cause an initial decrease in boar fertility and indirectly in its spermatozoa motility and concentration (Close, 1996). Day and night temperatures are higher during summer. Its effect on decreasing boar spermatozoa quality is probably smaller during the period of lower temperatures with a warm and comfortable straw bedding (Cap, 1995; Kozdrowski and Dubiel, 2004). Seasonal variations and individual variability are also noticeable to decrease boar spermatozoa quality. Seasonal changes in fertility of Kolbroek boars are poorly understood.

Table 2.1: Effect of season on semen characteristics of boar fertility

	Season				
Semen characteristics	Summer	Autumn	Winter	Spring	References
Volume ejaculate	327.0	344.7	310.0	293.8	Petrović *et al.* 2016
Number of ejaculates (n)	21	31	32	30	Górski *et al.* 2017
TNS ($x10^9$)	260.0	213.6	309.9	289.9	Petrović *et al.* 2016
Motility (%)	85.2	80.0	88.2	86.1	Petrović *et al.* 2016
Total abnormalities (%)	13.2	21.0	10.2	18.8	Petrović et al. 2016
Primary abnormalities (%)	3.6	10.9	1.1	10.5	Petrović et al. 2016
Secondary abnormalities (%)	9.6	10.1	9.1	8.3	Petrović et al. 2016
Number of produced doses	31.04	28.35	33.43	31.70	Petrović et al. 2016

TNS =total number spermatozoa

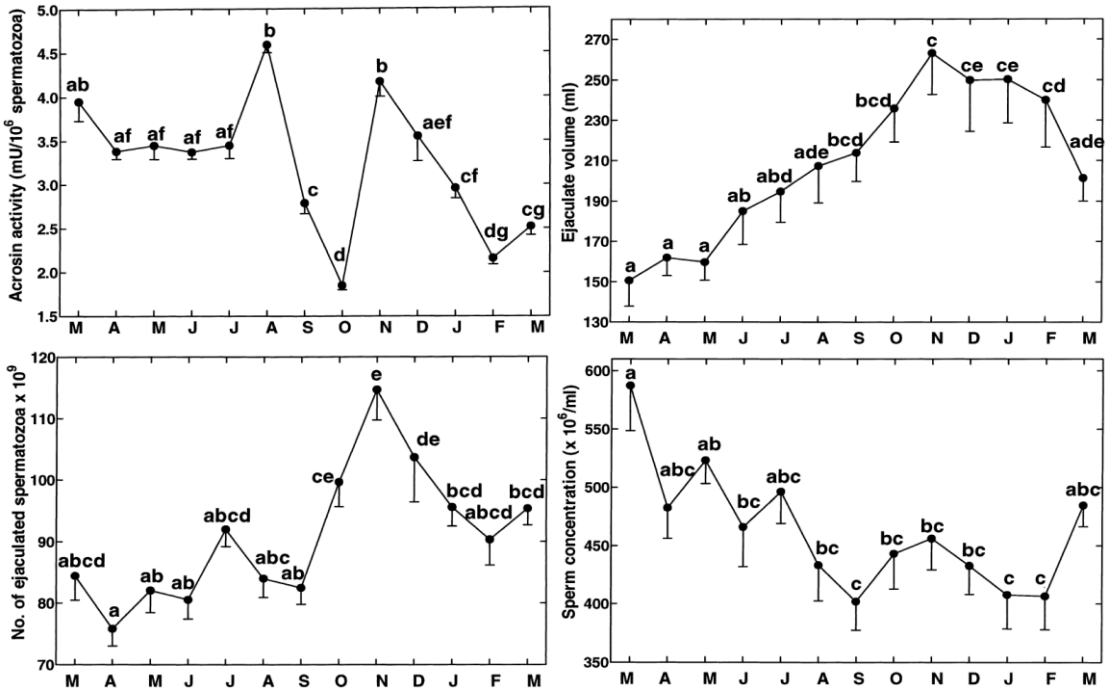

Figure 2.5: Effect of season on spermatozoa acrosin activity and number of spermatozoa in ejaculateand spermatozoa concentration of of boar semen

Source: Ciereszko *et al*. (2000).

2.4.7 Rhythm of semen collection

Semen collection from boars in AI centres is performed approximately twice per week (Vyt *et al.*, 2007). A high frequency of collection has a negative effect on semen quality because a spermatozoon is forced to rapidly pass from caput to cauda of the epididymis thus having insufficient time for epididymal maturation (Strzezek *et al.*, 1995). Submitting boars to collection four days in a row also affected the re-absorption/ secretion pattern of fluids in the lumen of epididymis (Pruneda *et al.*, 2005). This imbalance in the secretion of fluids resulted in an increase of abnormal spermatozoa and a reduction in spermatozoa motility. Goldberg *et al*. (2013) reported that the first part of the ejaculate (~25 ml) should be discarded because it does not contain spermatozoa and it may have a high bacterial count. Subsequently, the spermatozoa rich fraction is collected (40 and 100 mL) which contains 80 and 90 % of all spermatozoa in the ejaculate. The last part of the ejaculate is again a clearer, watery fluid which need not be collected as it contains few spermatozoa and is mainly secretions of the vesicular, prostate, and, towards the end of the ejaculation, bulbourethral glands. The effect of semen collection frequency on total number of spermatozoa motility and acrosion activity of boar is shown in Figure 2.6. Pruneda *et al*. (2005) reported that the boars collected twice a day for four consecutive days had more motility, proximal droplets, more head and tail abnormalities and lower motility than control boars collected once every other day in the same period. Frangeî *et al.* (2005) reported that the spermatozoa quality decreases with increasing collection frequency and is most pronounced at the collection frequency of seven times per week. Strzezek *et al*. (1995) reported that high collection frequencies lead to decreased spermatozoa motility, concentration, total spermatozoa and increased percent abnormal spermatozoa and spermatozoa with damaged membranes. There is no information in Kolbroek pigs.

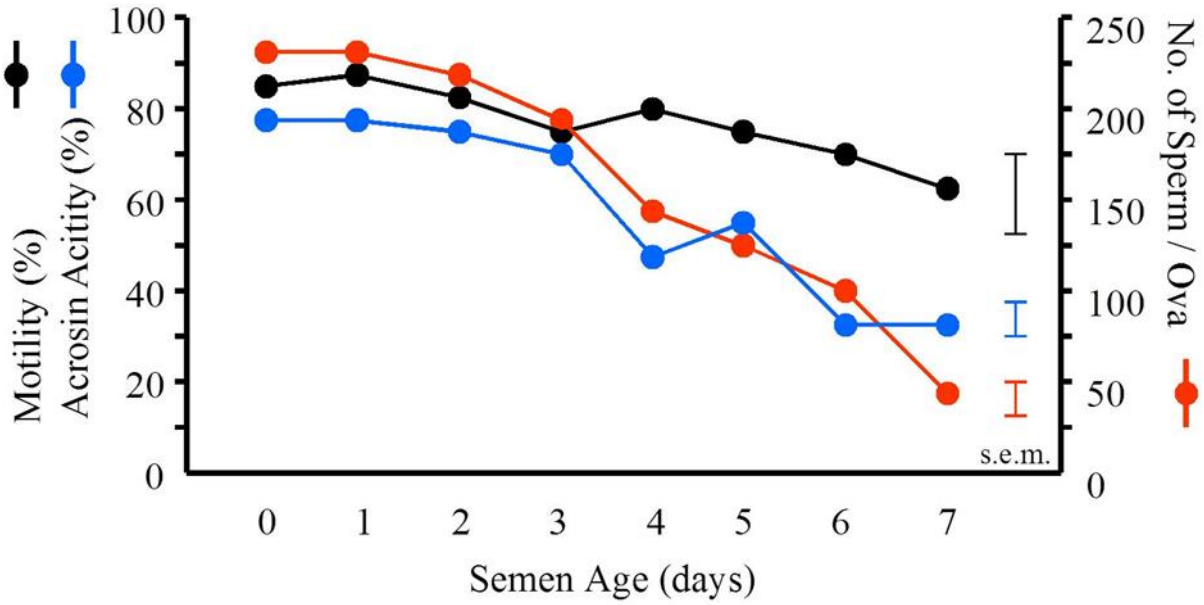

Figure 2.6: Effect of semen collection frequency on total number of spermatozoa motility and acrosion activity of boar

Source: Flowers (2017).

2.4.8 Social contact with other pigs

Pigs are social animals that under free-ranging conditions live in groups of approximately eight individuals (Landsberg, 2015). Although adult boar is housed in individual pens, group housing of growing boars is beneficial for subsequent reproductive performance. Groups of eight boars from 30 kg housed in pens of 4 × 4.3 m until they successfully completed two mountings, had on average stronger legs for jumping, higher libido, earlier accomplishment of the first mating and high spermatozoa counts compared to boars housed individually (Hacker *et al.*, 1994). Mature boars should not be kept together as they will fight and cause each other severe injuries. Umesiobi (2010) indicated that it is much easier to heat-check sows by fence-line boar contact instead of placing the boar in the sow pen for full boar contact. Fenceline contact with a boar is inadequate to stimulate puberty in most sows. Mature boar contact is needed when sows are

taken to a high stimulation area that only houses boars (Umesiobi, 2007). No information is available in Kolbroek pigs.

2.4.9 Accuracy of semen processing

Boar semen collection is usually performed by the gloved handed technique (Knox *et al.*, 2008). Polyvinyl gloves can be used; latex gloves should be avoided as these are toxic for the spermatozoa. The end of the penis is grabbed firmly with a gloved hand and the collection process is initiated with firm pressure to the spiral end of the penis with the hand so that the penis cannot rotate. This process imitates the pressure applied by the corkscrew shape of the sow's cervix. A pre-warmed (38 ℃) collection container is used to avoid rapid cooling of the ejaculate (Maes *et al.*, 2011). The top of the container is covered with cheesecloth to filter out gel portion of the semen. The first part of the ejaculate (pre-spermatozoa) should be discarded. The spermatozoa-rich fraction should have collected (40 to 100 ml). It is chalky in appearance and contains 80 and 90 % of all spermatozoa cells in the ejaculate. The ejaculation lasts up to 5 to 8 min, but may continue up to 15 min. About 100 to 300 mL of semen is routinely collected.

After collection, the filter with gel should be discarded and the collection container should be placed in warm water. Spermatozoa motility and vitality will be only being retained for few hours (Johnson *et al.*, 2000). To prolong spermatozoa survival, their metabolic activity should be inhibited by chemical inhibitors or by lowering the temperature and, therefore, the ejaculate needs to be extended shortly after collection (Johnson *et al.*, 2000). Compared to semen of other animal species, boar spermatozoa are susceptible to temperatures below 15 ℃, due to a different composition of the phospholipids in their membrane (De Leeue *et al.*, 1990). The temperature of the ejaculate of collection is approximately 37 ℃ and is between 32 and 35 ℃ at arrival in the laboratory where it is processed (Waberski, 2009). Fast cooling of the ejaculate

from body temperature to temperature below 15 ℃ result in lipid phase separation that will alter the spermatozoa membrane permeability with subsequent loss of spermatozoa vitality (Johnson *et al.*, 2000). These changes in membrane permeability result in a calcium influx into the spermatozoa that would stimulate capacitation-like changes (Petrunkina *et al.*, 2005).

2.5 Effect of selenium and zinc supplementation on growth performance, carcass characteristics and visceral organ weight

2.5.1 Growth performance

The body weight of a pig is an important indicator of its growth, health and readiness to go to the market (Wang *et al.*, 2008). Rahman *et al.* (2008) and Zhang *et al.* (2014) reported that zinc supplementation increased ADG, ADFI and FCR in pigs. Liu *et al.* (2011) reported that zinc supplementation led to an increased ADG of broilers. Kim and Mahan (2001) reported the increasing of dietary level of selenium increased body weights, daily gain and gain: feed ratio in growing-finishing pigs. Daily feed intake declined as the dietary selenium level increased in growing-finishing pigs (Kim and Mahan, 2001). Pigs fed increasing of dietary selenium had an increase in ADFI (Kim *et al.*, 2006). Cao *et al.* (2014) reported that the increase of selenium supplementation increased FCR in pigs. Wang *et al.* (2012) reported that zinc supplementation increased ADFI, ADG and FCE in pigs. Zhang *et al.* (2013) reported that ADFI decreased and the G/F increased) in pigs fed on diet with a higher zinc content compared with those that received the control diet. Dietary selenium supplemention increased ADFI and FCE in pigs (Júnior *et al*., 2017). However, there is no information on the interaction of selenium × zinc on the growth performance of pigs

2.5.2 Carcass characteristics

The slaughter weight, carcass weight, eviscerated weight, and breast and leg muscle weight were increased with increasing dietary zinc levels of Pekin ducks (Wen *et al.,* 2018). Son *et al.,* 2018) reported that increasing of selenium concentration linearly increased the weight of liver and kidneys. Wen *et al*. (2018) reported that zinc supplementation decreased the drip loss in breast meat of ducks. As the dietary zinc level increased, the drip loss decreased in ducks (Wen *et al.,* (2018). Increasing selenium levels increased the drip loss of pigs (Ortman and Pehrson, 1998). Zhan *et al*. (2006) and Bobcek *et al*. (2004) reported that selenium supplementation reduced loin drip loss. Feldpausch *et al.* (2015) reported that zinc supplementation improved carcass characteristics, warm carcass, cold carcass, dripm loss and dressing percentage in pigs. Increasing dietary levels of selenium also decreased backfat thickness in pigs (Daun and Akesson. 2004). Li *et al.* (2011) reported that selenium supplementation decreased drip loss and intramuscular fat percentage (Bobcek *et al.,* 2004). Percent drip loss as added dietary selenium concentration increases in pigs fed diets containing organic or inorganic selenium (Mateo *et al.,* 2007) are shown in Figure 2.7. Mahan *et al.* (1999) reported that 0.3 mg/kg of organic selenium in diets with an indigenous selenium content of 0.06 mg/kg reduced drip loss and improved compared to inorganic selenium supplementation in pigs. Both Zhan *et al.* (2006) and Bobcek *et al.* (2004) reported that organic selenium reduced loin drip loss in pigs. Therefore, it is important to investigate carcass traits of Large White × Landrace and Kolbroek pigs subjected to inclusion levels of selenium × zinc interaction.

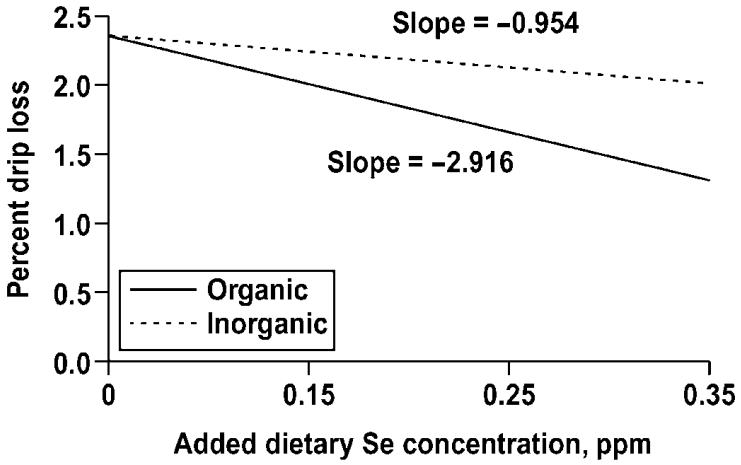

Figure 2.7: Percent drip loss as added dietary selenium concentration increases in pigs fed diets containing organic or inorganic selenium

Source: Mateo *et al.* (2007).

2.5.3 Visceral organ size

Schell and Kornegay (1996) reported that concentration of zinc in liver was increased with of dietary supplementation zinc of pigs. Selenium supplementation increased organ weights of pigs (Goehring *et al.*, 1984; Kim and Mahan, 2001; Kim and Mahan, 2001; Mateo *et al.*, 2007). However, the zinc concentration in the liver, intestine and kidney plays a key role in the maintenance of zinc in grower finisher pigy (Revy *et al.*, 2003). However, the mechanism behind these phenomena is poorly understood. Jahanian *et al*. (2008) reported that increasing zinc supplemental levels from 40 to 80 mg/kg of zinc increased liver weight percentage in broiler chicks. Speight *et al.* (2012) reported that boars injected with 0.33 mg/kg selenium every 14 days increased selenium concentrations in serum, kidney, liver, heart, skeletal muscle. Wen *et al.* (2018) reported that the weight of liver was linearly increased with zinc supplementation in ducks. Case and Carlson (2002) reported that as levels of zinc increased, the weight of liver and kidney increased. The relative liver weights were increased by increasing the dietary levels of zinc in pigs (Wang *et al.*, 2012). However, studies on interaction of selenium and zinc supplementation on intestine, liver, kidneys, spleen, liver and lungs organs in pigs are scarce.

2.7 Summary

Information regarding the genotype × selenium × zinc supplementation in pigs is scarce. The effect of selenium and zinc supplementation of pig genotypes on growth and reproductive performance, libido, testosterone hormones, semen quality and carcass traits needs to be investigated. Selenium can effectively prevent health problems in pigs. Selenium and zinc may exert beneficial effects on spermatogenesis in pigs.

Fertile boars should expreses the highest level of sexual behaviour and produce the greatest number of ejaculate, the best quality of ejaculate and the high number of insemination doses. Boar spermatozoa are sensitive to peroxidative damage due to high content of unsaturated fatty acids. Little, if any, is known about the effect of selenium × zinc interaction on boar spermatozoa quality of Large White × Landrace and Kolbroek boars. Therefore, the broad objective of the study was to determine the response in boar fertility to increasing selenium and zinc supplementation of Large White × Landrace and Kolbroek boars.

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Chapter 3

Effect of selenium × zinc interaction on growth performance of Large White × Landrace and Kolbroek boars

Abstract

The current study sought to determine the effect of genotype × selenium × zinc interaction on growth performance of Large White × Landrace (LW × LR) and Kolbroek boars. The boars were assigned to four experimental groups in a 2 × 2 × 2 factorial arrangement in a completely randomized design with six boars per treatment. Forty-eight boars of 7 - 8-month age and with average body weight of 44.5 ± 0.5 kg for LW × LR (n=24) and 41.2 ± 1.9 kg for Kolbroek (n=24) were used. Six boars of each genotype were fed one of four treatment combinations with high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) and low selenium (0.26 mg/kg) and low zinc (0.35 mg/kg) namely: high selenium high zinc (HSHZ), high selenium low zinc (HSLZ), low selenium high zinc (LSHZ) and low selenium low zinc (LSLZ). Weekly body weights and average daily feed intake (ADFI) were measured. Average daily gain (ADG) and feed conversion ratio (FCR) were estimated. Selenium and zinc supplementation did not increase final weight, ADG, ADFI and FCR of both Large White × Landrace and Kolbroek pigs ($P>0.05$). The were no interaction amoung Se, Zn and genotype no effect on growth performance ($P > 0.05$). In conclusion, selenium and zinc supplementation did not improve growth performance of pigs.

Keywords: pigs, average daily gain, average daily feed intake, feed conversion ratio, final weight, minerals

3.1 Introduction

Kolbroek pigs in southern Africa are mainly owned by economically vulnerable groups in marginal areas where they are used as a source of food, income and security (Halimani *et al.*, 2012). This is mainly because indigenous pig genotypes are well adapted to the backyard and scavenging production systems to harsh environments and handling conditions, which may influence pork quality (Hoffman *et al.*, 2005). There is a need to determine the possibility of using South African indigenous pig genotypes in the mainstream pigs production by exploiting their positive attributes.

Indigenous pigs are able to utilise fibrous feeds better than improved European genotypes (Kanengoni *et al.*, 2002). Mushandu *et al.* (2005) indicated that indigenous pigs are better able to utilise high-tannin red sorghum much than Large White pigs. Zinc is an indispensable component of several enzymes that participate in the synthesis and degradation of proteins, lipids, carbohydrates and nucleic acids (O'Dell, 2000) and also involved in the metabolism of other micronutrients. Adewol *et al.* (2016) found high amount of zinc is usually added in feed of growing pigs and it (zinc) had great effects on improving growth performance and alleviating diarrhoea (Hahn and Baker 1993). Supplementing zinc to livestock enhances growth, reproduction and immunity (Lin *et al.*, 2013). Altering pig nutrition strategies providies opportunities not only for improvement of animal health and productivity but also for production of enriched pork, and other foods that improves human diets (Tian *et al.*, 2006; Surai and Fisinin, 2015). The NRC (1998) established selenium requirements at 0.1 mg/kg, based on experiments of selenium retention in tissues of growing-finishing pigs. Selenium also activaties the glutathione peroxidase (GSH-Px), the anti-oxidation enzyme responsible for preventing peroxidation of body tissues (Tian *et al.*, 2006).

Zinc and selenium are essential micro-minerals that optimise growth and reproduction and stimulate immune responses in pigs (Close, 2003; Roberts *et al.,* 2002; Lee *et al.,* 2016). They are, however, expected to be limiting in diets fed to indigenous pigs. Supplementation of selenium in growing/finishing pigs lead to significant increase in the muscle tissue (Close, 2003). There is no information on genotype × selenium × zinc supplementation on growth performance of LW × LR and Kolbroek pigs. However, Kim *et al.* (2004) reported that selenium supplementation increased serum and tissue levels in growing-finishing pigs. Studies on the interactions of these critical micro-nutrients on pig performance, are little known and none have been done on the Kolbroek. Hence, the objective of study was to determine the selenium × zinc interactions on growth performance of LW × LR and Kolbroek boars. It was hypothesized that there is interaction between selenium and zinc on final weight, ADFI, ADG and FCR of LW × LR and Kolbroek pigs.

3.2 Materials and methods

3.2.1 Experimental site

The study was conducted at the Agricultural Research Council-Animal Production Institute (ARC-API), South Africa. The ARC-API campus is located at 25^0 55′ South; 28^0 12′ East and is located in the highveld and situated at an altitude of 1525 m above sea level. Experimental boars were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (Ref: APIEC16/037 from November 2016 to February 2017.

3.2.2 Experimental pigs and housing

Forty-eight mature pigs of 7 to 8-month age and with 44.5 ± 0.5 kg average body weight of LW × LR (n = 24) and Kolbroek (n = 24) with average body weight 41.2 ± 1.9 kg were used

for the study. The boars were allowed to acclimatize for two weeks on the allocated diets which were offered at 2 kg per pig and water was supplied. The pens were cleaned daily and the pigs were dipped in a Triatix® pig pour-on (Amitraz) every two weeks against ectoparasites and were dewormed once a month against endo-parasites using Valbazen® (Albendazole). The pens for the Large White × Landrace and Kolbroek boars measured 2 × 1.5 m in environmentally controlled houses with temperature ranging from 22 to 25°C.

3.2.3 Experimental design and diets

Twenty-four Kolbroek and 24 LW × LR boars were used in a 2 × 2 × 2 (genotype × zinc and selenium concentration × high and low) factorial arrangement. Six pigs of each genotype were randomly assigned to four experimental diets containing either high or low levels of selenium and zinc. The four diets were: low selenium (0.26 mg/kg) and low zinc (0.35 mg/kg) (LSLZ); high selenium (0.65 mg/kg) and high zinc (0.74 mg/kg) (HSHZ); low selenium (0.26 mg/kg) and high zinc (0.74 mg/kg) (LSHZ); and high selenium (0.65 mg/kg) and low zinc (0.35 mg/kg) (HSLZ).

Table 3.1 shows the ingredient and chemical composition of the diets. A total mixed ration was formulated to supply14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg DM and 11.6 g lysine/kg which met the requirements of growing pigs (NRC, 1998). The dry matter (DM), ash, crude protein (CP), ether extract (EE), zinc, selenium, and acid detergent fibre (ADF) were analysed following the procedures of the Association of Official Analytical Chemists (2005) and neutral detergent fibre (NDF) was determined following the procedures of van Soest (1963).

3.2.4 Measurements

3.2.4.1 Growth performance

Growth performance variables were determined every week for an experimental period of six months following an adaptation of two weeks. The amount of feed supplied to each pig was weighed and recorded daily. To calculate feed intake, the amount of feed inside the feeding trough after every seven days was subtracted from total feed supplied for seven days and feed remains were weighed and recorded. Pigs were weighed every week. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for each group of pigs.

Table 3.1: Ingredient and chemical composition of the diets

Ingredient (%)	Diets[1]			
	HSLZ	HSHZ	LSHZ	LSLZ
Yellow maize	62.25	62.25	62.25	62.25
Hominy chop	8.64	8.64	8.64	8.64
Feed lime	0.27	0.27	0.27	0.27
Monocalcium phosphate	2.95	2.95	2.95	2.95
Soya bean oil cake	24.69	24.69	24.69	24.69
Vitamin mineral premix[3]	0.20	0.20	0.20	0.20
Salt	1.00	1.00	1.00	1.00
Zinc	0.35	0.74	0.74	0.35
Selenium	0.65	0.65	0.26	0.26
Chemical composition analysis (%)				
Dry matter	90.45	90.65	90.28	90.22
Protein	16.74	16.74	16.17	16.72
NDF	20.17	21.47	19.95	19.59
ADF	4.58	4.94	4.41	4.96
Crude fibre	3.31	3.08	3.31	3.59
Digestible energy MJ/kg	20.31	20.71	20.44	20.21
Phosphorus	0.87	0.72	0.78	0.89
Calcium	0.80	0.60	0.76	0.68
Zinc	0.04	0.06	0.06	0.04
Selenium	0.006	0.006	0.001	0.001

[1]HSLZ = high selenium, low zinc; HSHZ = high selenium, high zinc; LSHZ = low selenium, high zinc and LSLZ = low selenium, low zinc

[2]NDF = neutral detergent fibre, ADF = acid detergent fibre

[3]The following minerals;4.5 mg vitamin B2, 0.03 mg vitamin B12, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous.

3.2.5 Statistical analyses

The genotype × selenium × zinc interactions on ADG, ADFI, and FCR were performed using GLM procedures in SAS version 9.3 (1999). A 5 % significance level was used. The model used was:

$Y_{ijkl} = \mu + S_i + Z_j + G_k + (S \times Z)_{ij} + (S \times G)_{ik} + (Z \times G)_{jk} + (S \times Z \times G)_{ijk} + E_{ijkl}$

Y_{ijkl} = ADG, ADFI, FCR and final weight.

μ = is the overall mean common to all observations

S_i = Selenium level (i = LS, HS)

Z_j = Zinc level (j = LZ, HZ).

G_k =Genotype (k = Kolbroek, Large White × Landrace)

$(S \times Z)_{ij}$=selenium × zinc is interaction

$(S \times G)_{ik}$ =the interaction of Selenium × genotype

$(Z \times G)_{jk}$= the interaction of Zinc × genotypes

$(S \times Z \times G)_{ijk}$= the interaction of selenium × zinc × genotype

E_{ijkl}= the residual error.

3.3 Results

3.3.1 Effect of genotype, selenium and zinc supplementation on growth performance

The effects of the different levels of zinc and selenium supplementation on growth performance of the LW × LR and Kolbroek boars are shown in Table 3.2. There was no genotype × zinc × selenium interactions, zinc × selenium interactions, zinc and selenium effect on final body weight (FW), ADFI and FCR in both genotypes ($P > 0.05$). There were, however, genotype differences ($P < 0.05$) in FW, ADFI and FCR of the LW × LR and Kolbroek boars. The LW × LR had higher ($P < 0.05$) FW, ADFI and FCR than the Kobroek boars ($P < 0.05$).

Table 3.2: Effects of different levels of zinc and selenium supplementation on the growth performance of the Large White (n = 24) × Landrace and Kolbroek boars (n = 24)

Genotypes[1]	Diets[3]		Measurements				
	Selenium	Zinc	IW	FW	ADFI	ADG	FCR
Kolbroek	High	High	40.1[a]	74.2[b]	1.52[a]	0.37[a]	4.1[a]
	High	Low	40.1[a]	73.6[b]	1.51[a]	0.37[a]	3.9[a]
	Low	High	40.0[a]	73.9[b]	1.50[a]	0.39[a]	3.9[a]
	Low	Low	40.0[a]	74.0[b]	1.52[a]	0.36[a]	4.2[a]
LW × LR	High	High	45.2[b]	81.6[a]	1.88[b]	0.41[b]	4.5[b]
	High	Low	45.0[b]	81.2[a]	1.92[b]	0.40[b]	4.8[b]
	Low	High	45.0[b]	82.1[a]	1.91[b]	0.41[b]	4.6[b]
	Low	Low	44.9[b]	81.2[a]	1.92[b]	0.40[b]	4.8[b]
	SEM		0.12	0.41	0.017	0.009	0.14
P-value	Genotype		***	***	***	***	***
	Selenium		NS	NS	NS	NS	NS
	Zinc		NS	NS	NS	NS	NS
	Se × Zinc		NS	NS	NS	NS	NS
	Genotype × Se		NS	NS	NS	NS	NS
	Genotype × Zinc		NS	NS	NS	NS	NS
	Genotype × Se × Zinc		NS	NS	NS	NS	NS

[ab] Values with different superscripts in a column differ significantly ($P < 0.05$), *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and NS not significant

[1]LW × LR= Large White × Landrace, n = 6 per diet and KB = Kolbroek, n = 6 per diet

[2]IW=initial weight, FW =final weight, ADFI = average daily feed intake, ADG = average Daily gain, FCR = feed conversion ratio

[3]low (se – 0.26 mg/kg) low; zn – 0.35 mg/kg) (LSLZ); low (se – 0.26 mg/kg) high zn 0.74mg/kg) (LSHZ); high (se – 0.65 mg/kg low zn – 0.35 mg/kg) (HSLZ) and high (se – 0.65 mg/kg; high zn 0.74mg/kg) (HSHZ).

3.4 Discussion

It was hypothesised that FW, ADFI, ADG and FCR are affected by inclusion levels of selenium and zinc supplementation in both genotypes. Tian *et al*. (2006) reported that the different levels of selenium supplementation did not influence growth performance in growing-finishing pigs. The finding showed that selenium and zinc supplementation did not influence ADFI of LW × LR and Kolbroek boars. The LW × LR crosses consumed more feed than Kolbroek boars. The LW × LR boar were expected to consume more feed than the Kolbroek boar because of their bigger body size and gut capacity (Whittemore *et al.,* 2003; Thacker and Haq, 2009). Tian *et al*. (2006) reported increases in ADFI in Landrace × Yorkshire × Large White pigs supplemented with selenium. As the dietary selenium increased, the ADFI increased in Large White × Landrace × Pietrain pigs (Jlali *et al.*, 2014; (Jang *et al.*, 2010). The greatest increases in the ADFI of Duroc × Landrace × Large White pigs supplemented with zinc (Wang *et al*., 2012). The ADFI increased as levels of zinc supplement increased (Bergeron *et al.,* 2019). Guo-jun *et al.* (2009) also reported that increasing zinc supplementation increased ADFI of Duroc× Landrance × Yorkshire pigs. Hernandez *et al.* (2008) reported that increasing selenium and zinc supplementation increased ADFI of Large White × Landrace pigs. There was a tendency for ADFI to increase with zinc supplementation of PIC pigs (Carpenter *et al.,* 2016). Coble *et al.* (2013) reported that adding zinc in pig diets decreases ADFI in finishing pigs. (Paulk *et al.*, 2015). However, studies on interaction of selenium and zinc supplementation on ADFI in pigs are scarce (Dobrzanski, 2003). In addition, there is no information on the effects on the interaction of selenium × zinc on the ADFI of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that future research should explore these aspects.

Unlike Kolbroek boars, LW × LR boars on low selenium had lower ADG than those on high selenium diets irrespective of the zinc levels. Selenium and zinc supplementation had no effects

on ADG in LW × LR and Kolbroek pigs. Radovic *et al.* (2017) reported that the Mangalitsa pigs had lower ADG than crossbreed pigs. Nevrkla and Václavková. (2018) reported that the local Prestice Black-Pied pigs had lower ADG compared to Large White × Landrace pigs. Guo-jun *et al.* (2009) reported increases in ADG in pigs supplemented with increasing dietary levels of zinc. Yang *et al*. (2010) reported that increasing selenium increased ADG of pigs. The greatest increases in ADG was observed in Duroc × Landrace × Large White pigs supplementated with increasing levels of zinc (Wang *et al*., 2012). Average daily gain increased as levels of dietary selenium increased in Large White × Landrace × Pietrain pigs (Jlali *et al.,* 2014). Zinc supplementation increased ADG (Bergeron *et al.,* 2019). Improvement in weight gain due to zinc supplementation was also reported by (Martinez *et al.* (2018). The ADG increased as levels of dietary levels of zinc supplementation increased (Zhang *et al*., 2014). Hernandez *et al.* (2008) reported that increasing selenium and zinc supplementation increases ADG in Large White × Landrace pigs. The ADG increased with on increased in zinc supplementation (Carpenter *et al.,* 2016). Coble *et al.* (2013) reported that pigs fed added zinc had decreased ADG in finishing pigs. There was an increased ADG in pigs fed diets with added zinc in finishing pigs (Paulk *et al.,* 2015) (Reffett *et al.,* 1986). The finding that the ADG in Kolbroek and LW × LR boars responded positively to selenium supplementation unlike Kolbroek boars. There is no information on the interaction of selenium and zinc on the ADG of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that research should further explore these issues.

The inclusion levels of selenium and zinc supplementation did not affect FCR of LW × LR and Kolbroek boars. Increasing of selenium and zinc does not increases FCR of LW × LR and Kolbroek boars. There is no evidence available on interaction of selenium × zinc influence the FCR of Large White × Landrace and Kolbroek pigs. The finding that the FCR in Kolbroek and

LW × LR boars responded positively to selenium supplementation. There was no effect of genotype on FCR of both LW × LR and Kolbroek boars. As expected, Large White × Landrace had higher FCR than for the Kolbroek pigs. Kanengoni *et al*. (2004) reported that the lower food conversion efficiency (PCE) that was found for the Kolbroek pigs as compared to the crossbred pigs, suggesting that Kolbroek pigs are early maturing, and thus, deposit body fat much faster than imported pigs leading to lower food conversion efficiency. An increase in the FCR was observed in Duroc × Landrace × Large White pigs supplementated with zinc (Wang *et al*., 2012). As the dietary selenium increased, the FCR increased in Large White × Landrace × Pietrain pigs (Jlali *et al.*, 2014). The FCR increased as levels of dietary selenium increased (Jang *et al.*, 2010). Jondreville *et al.* (2007) reported that an improved FCR due to zinc supplementation. Feed converaion ratio increased as levels of dietary levels of zinc increased (Zhang *et al*., 2014). Feed conversion ratio are improved as levels of zinc supplementation increased (Bergeron *et al.*, 2019). Guo-jun *et al.* (2009) reported that increasing zinc supplementation improved FCR in Duroc × Landrance × Yorkshire pigs. (Reffett *et al.*, 1986). Therefore, further rearch should investigate FCR of Kolbroek and LW × LR pigs subjected to interaction of selenium and zinc supplementation.

Selenium and zinc supplementation did not influence final weight (FW) of LW × LR and Kolbroek boars. There was an effect of genotype on final weight (FW). The highest body weight was observed in LW × LR boars supplemented with low selenium high zinc inclusion. The Kolbroek is an early maturing breed that grows slower than improved breeds, therefore, the lower BW was expected. The body weight of pigs is an important indicator of its growth, health, reproductive efficiency and readiness for slaughter (Anglart, 2016). The LW × LR boars consumed more feed per metabolic weight than the Kolbroek pigs (Kanengoni *et al.*, 2014). The LW × LR pigs would naturally consume more feed than Kolbroek pigs because of their

bigger body size and gut capacity (Whittemore *et al.*, 2003). Feldpausch *et al.* (2014) reports that increases in FW in growing-finishing pigs supplement with increasing levels of dietary selenium. As zinc is not stored in the body, inadequate dietary intake of zinc and inhibitors of zinc absorption are most likely the common causative factors for suboptimal zinc status within the body (Jlali *et al.*, 2014). Zhang *et al*. (2014) reported that the FW increased with increasing dietary levels of zinc supplementation in pigs. As dietary selenium increased, the FW increased in Large White × Landrace × Pietrain pigs. The finding that the FW in Kolbroek and LW × LR boars responded positively to selenium supplementation unlike Kolbroek boars. There is no evidence available on interaction of selenium × zinc influence the FW of Large White × Landrace and Kolbroek pigs. Therefore, further rearch should investigate FW of Kolbroek and LW × LR pigs subjected to interaction of selenium and zinc supplementation.

3.5 Conclusions

There were genotype effects on growth performance of boars. There were no effects of selenium and zinc supplementation on growth performanceof both White × Landrace and Kolbroek boars. Increasing levels of selenium and zinc supplementation did not increase the final weight, ADG, ADFI and FCR of both genotypes. This implies that selenium and zinc supplementation may not be essential mineral in influencing growth performance of pigs.

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Chapter 4

Interaction of genotype, selenium and zinc supplementation on sexual behaviour, semen volume and testosterone levels in boars

Abstract

The objective of the current study was to assess the effect of genotype × selenium × zinc on sexual behaviour, semen volume and plasma testosterone concentration in Large White × Landrace (LW × LR) and Kolbroek boars. A total of 48 boars (24 LW × LR and 24 Kolbroek) at 7 to 8-months of age and average live weight of ± 41.2 Kolbroek and ± 55 LW × LR were used in the study. Boars were assigned to four treatments of six boars each experimental groups in a 2 × 2 ×2 factorial design with eight boars per treatment: (HSHZ) high selenium (0.65 mg/kg) high zinc (0.74 mg/kg) (n=6), (HSLZ) high selenium (0.65 mg/kg) low zinc (0.35 mg/kg) (n=6), (LSHZ) low selenium (0.26 mg/kg) high zinc (0.35 mg/kg) (n=6) and (LSLZ) low selenium (0.26 mg/kg) low zinc (0.35 mg/kg) were used to feed the pigs for six months. Boars were trained daily to mount a dummy sow for semen collection for four weeks. Semen was collected by using the gloved hand technique twice per week for six months. Blood samples were collected from each boar monthly to determine testosterone concentrations. Kolbroek boars had longer time to mounts with penis exposed (TMNP), time mount without penis exposed (TMWP) and duration of ejaculation (DE) ($P< 0.05$) than LW × LR boars. Kolbroek boars fed on the HSHZ diet had lower TMNP ($P < 0.05$) than those fed on the HSLZ diet ($P < 0.05$). Kolbroek boars fed on the LSLZ diet had higher TMWP ($P < 0.05$) than those

fed on the LSHZ diet. The LW × LR boars fed on the LSLZ diet had higher DE ($P < 0.05$) than LW × LR boars fed on LSHZ and HSHZ diets. The LW × LR boars fed on HSHZ and HSLZ had higher ejaculation volume and testosterone concentrations than LW × LR boars fed LSHZ and LSLZ diets ($P < 0.05$). In conclusion, selenium and zinc supplementation did not increase testosterone concetration and DE of Large White × Landrace and Kolbroek boars. There were no effects of genotype on ejaculation volume of both genotypes.

Keywords: libido, semen volume, testosterone, minerals, mounting behaviour

4.1 Introduction

Although boars are bred primarily for traits of economic importance, it is necessary to take into account sex drive as an essential criterion for selecting. There are expected differences between genotypes and lines of pigs in libido and sexual behaviour, as well as duration of boar ejaculation which can be attributed to genetic and hormonal factors and para genetic impacts (Okere *et al*., 2005). Nutritional staus of boars is likely to influence sexual behaviour of pigs. Both deficiency and excess of dietary selenium influence reproductive problems in LW × Landrace pigs (Kohrle *et al.,* 2005). No work has been reported on the Kolbroek boars. These pigs are hardy, seemingly well adapted to harsh environmental conditions (Mapeka *et al.,* 2012; Swart *et al.,* 2010). They are better able to utilize fibrous feeds and are tolerant to endemic diseases and parasites. They, however, exhibit slow growth rates and inadequate meat production (Prolit, 2004). Halimani *et al*. (2012) reported that indigenous pigs in Southern Africa are usually kept among resource-poor households who reside in marginal areas and are vulnerable to cnages in natural environments. The small sizes of most indigenous pig populations increases the risk of inbreeding and the resultant loss of biodiversity (Halimani *et al*., 2012).

Clark (2007) indicated that the level of mounting among boars shows considerable variability within a population. Individual differences in mounting behaviour are related to increase the circulating sex hormones and onset of puberty. Differences in the performance of mounting may reflect a form of dominance behaviour (Cronin *et al.*, 2003; Rydhmer *et al.*, 2006; Fredriksen *et al.*, 2008). Mounting sexual behaviour of Kolbroek boars, therefore, needs to be understood to enhance fertility of the populations (Hintz *et al.*, 2013). There is limited information about the effect of zinc and selenium supplementation (Neek *et al*., 2011).

Selenium is a trace element that plays an important role in the health and reproduction performance. In pigs, selenium is required for the maintenance of male fertility, its deficiency causes reduced numbers of spermatozoa, an impairment of spermatogenesis, and reduces fertilization capacity (Maiorino *et al*., 1999). Selenium is protective and is a chain breaking anti-oxidant of peroxidase enzymes formed during cell metabolic processes (Kumar *et al*., 2011). Little work has been done on the importance of selenium and zinc supplementation on semen quality Kolbroek pigs. Boars that show a higher sexual stimulation, and respond to reacting more rapidly to the dummy sow, produce more spermatozoa (Szostak and Sarzyńska, 2011). Selenium also acts with different potency on six biochemical markers including, testosterone and epithelial cell deoxyribonucleic acid damage of pigs (Waters *et al.*, 2012).

The role of zinc in pigs, is for storage and secretion of hormones as well as in the effectiveness of receptor sites and end-organ responsiveness (Egwurugwu *et al.*, 2013). Zinc is essential in the secretion of in testosterone, growth hormone (GH), thyroid stimulating hormone (TSH), glucagon, insulin, follicle stimulating hormone (FSH), luteinizing hormone (LH) gonadotropin releasing hormone and adrenocorticotropic hormone (Alves *et al*., 2012). In boars, zinc

increases the efficiency of spermatogenesis and number of germ cells in the seminiferous tubules (Pizent *et al.*, 2003; Abdella *et al.*, 2015). Zinc also inhibits the aromatase that converts testosterone into excess luteinizing hormone (Debjit *et al.*, 2011). Selenium concentrations in plasma and tissues is also regulated by testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Surai, 2006), which also influences sexual behaviour of the boars. Inadequate zinc levels prevent the pituitary gland from releasing LH and FSH (Onah *et al*., 2015). Savić and Petrović (2015) reported that, in addition to monitoring libido, it is necessary to record the duration and time period of boar's semen collection. Libido is estimated as the reaction and time from the moment of allowing the boar into the place with the dummy sow and taking a mount, and the total time of copulation (Rydhmer *et al.*, 2010; Szostak and Sarzyńska, 2011).

Testosterone is produced by the interstitial cells of the Leydig cell and is responsible for male secondary sexual characteristics and spermatogenesis (Tilbrook and Clarke, 2001; Arthur *et al.*, 2006; Onah *et al.*, 2015). Testosterone is essential for the development and maintenance of sexual behaviour of boars (Behne *et al*., 1996; Mahmoud, 2012). No data are available on the effects of genotype × selenium × zinc concentrations and the relation to spermatozoa quality, libido and testosterone concentrations in L W × LR and Kolbroek boars. The objective of the current study was to determine the effect of inclusion level of selenium and zinc supplementation on testosterone concentrations, sexual behaviour and semen volume of LW × LR and Kolbroek boars. It was hypothesised that supplementating selenium and zinc interaction improves sexual behaviour, semen volume and blood testosterone concentration in both genotypes.

4.2 Materials and methods

4.2.1 Study site

The study was conducted Agricultural Research Council, Germplasm Conservation and Reproductive Biotechnologies Unit, Irene, South Africa. The ARC-Irene campus is located at 25˚55' South; 28˚12' East. The institute is located in the Highveld region of South Africa and situated at an altitude of 1525 meters above sea level. The study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Animals under the guidelines of the Agricultural Research Council, Animal Production Institute Animal Ethics Committee (APIEC/16/002).

4.2.2 Pig housing

Each boar was housed individually in a 1.54 × 0.8 m pens in an environmentally controlled house with the temperature ranging from 22 to 25 °C. Boars were fed in feeding troughs and water was accessible *ad libitum* through nipple drinkers per pig. Temperature was regulated by means of window controlled natural ventilation. The pens for the LW × LR and Kolbroek boars were measured on 2 m x 1.5 m in environmentally controlled houses with the temperature ranging from 22 to 25 °C. All the pens were cleaned daily and the pigs were dipped in Triatix® (Amitraz) every two weeks against ectoparasites and were dewormed once a month against endo-parasites using Valbazen® (Albendazole).

4.2.2 Pigs, diets and sampling

A total of 48 boars (24 LW × LR and 24 Kolbroek) at the age 7 to 8-months of and average body weight of ± ± 41.2 Kolbroek and ± 55 LW × LR were used for the study. Boars were assigned to four experimental groups in a 2 × 2 × 2 factorial design with six boars per treatment.

An environmental, facility and dietary acclimatisation period of two weeks was allowed for pigs before data collection. Water was available through low-pressure nipple drinkers. A total mixed ration was formulated to supply 14 MJ/kg digestible energy, 180 g crude protein (CP)/kg DM and 11.6 g lysine kg which meet the requirements of growing pigs (NRC, 1998) but varying in combinations of two concentrations of selenium and zinc at either low (Se – 0.26 mg/kg; Zn – 0.35 mg/kg) or high (Se – 0.65 mg/kg; Zn 0.74 mg/kg). The diets were as follows: low selenium low zinc (LSLZ); low selenium high zinc (LSHZ); high selenium low zinc (HSLZ) and high selenium high zinc (HSHZ). The LW× LR were and Kolbroek were each fed 2 kg per day until the end of the experiment. The composition of experimental diets with different concentrations of zinc and selenium fed to LW × LR and Kolbroek boars are described in Table 4.1.

Table 4.1: Ingredient and chemical composition of the diets

	Diets[1]			
Ingredient (%)	HSLZ	HSHZ	LSHZ	LSLZ
Yellow maize	62.25	62.25	62.25	62.25
Hominy chop	8.64	8.64	8.64	8.64
Feed lime	0.27	0.27	0.27	0.27
Monocalcium phosphate	2.95	2.95	2.95	2.95
Soya bean oil cake	24.69	24.69	24.69	24.69
Vitamin mineral premix[3]	0.20	0.20	0.20	0.20
Salt	1.00	1.00	1.00	1.00
Zinc	0.35	0.74	0.74	0.35
Selenium	0.65	0.65	0.26	0.26
Chemical composition analysis (%)				
Dry matter	90.45	90.65	90.28	90.22
Protein	16.74	16.74	16.17	16.72
NDF	20.17	21.47	19.95	19.59
ADF	4.58	4.94	4.41	4.96
Crude fibre	3.31	3.08	3.31	3.59
Digestible energy MJ/kg	20.31	20.71	20.44	20.21
Phosphorus	0.87	0.72	0.78	0.89
Calcium	0.80	0.60	0.76	0.68
Zinc	0.04	0.06	0.06	0.04
Selenium	0.006	0.006	0.001	0.001

[1]HSLZ = high selenium, low zinc; HSHZ = high selenium, high zinc; LSHZ = low selenium, high zinc and LSLZ = low selenium, low zinc

[2]NDF = neutral detergent fibre, ADF = acid detergent fibre

[3]The following minerals;4.5 mg vitamin B2, 0.03 mg vitamin B12, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous.

4.2.3 Training of boars and mounting behaviour

Boars in all groups were trained daily for semen collection with a dummy sow for two weeks. The sexual activity of the boars was assessed based on the time when the successive copulatory reflexes were released during manual semen collection (Wysokińska and Kondracki, 2014; Savić and Petrović, 2015). The sexual activities were selected for assessment and observation for time from the boar entering the arena until mounting the phantom, time from phantom mounting until the start of ejaculation, duration of ejaculation, total copulation time, number of climbs onto the phantom necessary to yield semen. The duration of sexual reflexes was determined using a stopwatch to one second. The measurements were conducted in the morning between 0830 and 1030 h.

4.2.4 Blood sampling and analyses

Blood was collected from 48 boars from the jugular vein once monthly at day 0 (baseline) and at 30, 60 and 90 days. Blood samples were collected into plain blood collection tubes which were transported to the laboratory in an icebox and centrifuged at 3000 g for 10 min. The harvested serum was kept in a freezer at –20°C until analysis of testosterone concentrations. Blood serum testosterone concentrations were determined using ELISA Kits (Immunotech, A Coulter Co., France) according to the manufacturer's instructions (Gado *et al.*, 2015). All serum samples for each hormone assay were run in duplicate in a single assay and samples

gave results parallel to the standard curve. The testosterone minimum detection limit was 0.04 ng/ml.

4.2.5 Statistical analyses

The interaction of selenium, zinc and genotype on semen quality, sexual behaviour and hormonal analysis were performed using SAS (1999). The GLM procedure was also used to determine the effect on interaction of selenium, zinc and genotype. A 5 % significance level were used.

The model used for sexual behaviour, time, duration ejaculation semen volume, and testosterone analysis was:

$Y_{ijkl} = \mu + S_i + Z_j + G_k + (S \times Z)_{ij} + (S \times G)_{ik} + (Z \times G)_{jk} + (S \times Z \times G)_{ijk} + E_{ijkl}$

Y_{ijkl} =sexual behaviour, time, duration, ejaculation, semen volume and plasma testosterone concentration

μ = is the overall mean common to all observations

S_i = Se level (i = LS, HS)

Z_j = Zinc level (j = LZ, HZ)

G_k =Genotypes (k = Kolbroek, LW × LR)

$(S \times Z)_{ij}$=Se × Zn interaction

$(S \times G)_{ik}$ =the interaction of selenium × genotype

$(Z \times G)_{jk}$= the interaction of zinc × genotype

$(S \times Z \times G)_{ijk}$= the interaction of selenium × zinc × genotype

E_{ijkl}= the residual error.

4.3 Results

4.3.1 Effect of selenium and zinc supplementation on sexual behaviour, ejaculation semen volume and testerone concentration

The effect of selenium and zinc supplementation on sexual behaviour of LW × LR and Kobroek boars are shown in Table 4.2. There was a genotype effect on testosterone ($P < 0.05$) concentrations. Kolbroek boars had higher TMNP, TMWP and DE values ($P < 0.05$) than LW × LR boars. Kolbroek boars fed on the HSHZ diet had lower TMNP ($P <0.05$) than those fed on the HSLZ diet. Kolbroek boars fed on the LSLZ diet had higher TMWP ($P < 0.05$) than those fed on the LSHZ diet. The LW × LR boars fed on the LSLZ diet had higher DE ($P < 0.05$) than boars fed on LSHZ and HSHZ diets ($P < 0.05$). The LW × LR boars fed on HSHZ and HSLZ diets had higher ejaculation volume and testosterone concentrations than LW × LR boars fed LSHZ and LSLZ diets ($P < 0.05$). There were no differences ($P > 0.05$) in ejaculation volume and testosterone concentrations among the Kolbroek boars fed on the different diets. Supplementation of selenium and zinc increased the number of mounts in both genotypes ($P < 0.05$).

Table 4.2: Effect of dietary levels of selenium and zinc on sexual behaviour, ejaculation volume and testosterone concentrations in Large White × Landrace (n = 24) and Kobroek (n = 24) boars

Genotype	Selenium mg/kg[1]	Zinc mg/kg[1]	Sexual behaviour[2]					Testosterone ng/ml
			TMNP, sec	TMWP, sec	EV, mL	DE, sec	NM	
LW × LR	High	High	165.2^{cd}	70.3^{c}	297.8^{a}	310.3^{b}	71.3	28.0^{c}
	High	Low	156.1^{d}	73.4^{c}	278.3^{a}	361.1^{bc}	78.3	34.5^{b}
	Low	High	173.9^{cd}	93.1^{bc}	210.0^{b}	350.7^{b}	86.5	7.1^{a}
	Low	Low	165.5^{cd}	89.5^{bc}	201.7^{b}	489.3^{ac}	95.7	7.5^{a}
Kolbroek	High	High	212.0^{bc}	110.6^{ab}	148.3^{c}	524.3^{a}	67.9	7.9^{a}
	High	Low	270.3^{a}	110.9^{ab}	110.8^{c}	504.2^{a}	75.7	9.4^{a}
	Low	High	254.5^{ab}	102.0^{bc}	109.2^{c}	484.5^{ac}	79.0	8.1^{a}
	Low	Low	255.4^{ab}	138.4^{a}	130.8^{c}	497.3^{a}	79.6	7.3^{a}
SEM			17.47	12.44	15.20	46.5	12.30	1.67
Pvalue	Genotype		***	***	***	***	NS	***
	Selenium		NS	NS	***	NS	NS	***
	Zinc		NS	NS	NS	NS	NS	NS
	Genotype × Se		NS	NS	**	NS	NS	***
	Genotype × zinc		NS	NS	NS	NS	NS	NS
	Selenium ×zinc		NS	NS	NS	NS	NS	NS
	Genotype × Se × zinc		NS	NS	NS	NS	NS	NS

[abcd] Values with different superscripts within a column differ significantly P < 0.05 ******* P < 0.001, ****** P < 0.01, *****P<0.05 and NS = not significant,

[1]HSLZ = high selenium, low zinc; HSHZ = high selenium, high zinc; LSHZ = low selenium, high zinc and LSLZ = low selenium, low zinc,

[2]NM = number of mounts, EV = ejaculation volume, TMWP = time mount without penis exposed, TMNP = time mounts with penis exposed; DE = duration of ejaculation

4.4 Discussion

Very few studies reported the interaction of selenium and zinc supplementation on sexual behaviour and hormonal patterns in boars. It was hypothesised that selenium and zinc supplementation had effect on sexual behaviour, semen volume and testosterone concentration of the LW × LR and Kolbroek boars. Selenium deficiency has been linked to reproductive problems in rats, mice, chickens, pigs, sheep, and cattle (Dorostkar *et al*., 2012). Selenium supplementation has been reported to improve reproductive performance in sheep and mice (Mohammad, 2012). However, little, if any, information was available on the interaction of selenium and zinc supplementation on sexual behaviour, semen volume and hormonal levels in LW × LR and Kolbroek pigs.

The finding that showed that the Kolbroek boars had higher TMNP, TMWP and DE values than LW × LR boars was unexpected. Reproductive performance of Kolbroek pigs in Southern Africa is known to be low. Kolbroek reach maturity early compared to Large White (Holness and Smith, 1973). Sexual desire exemplified by reaction time is an important aspect of male reproductive function (Umesiobi and Iloeje, 1999). It was observed that the TMNP, TMWP and DE were influenced by selenium and zinc inclusion in the diet. At low levels of selenium and zinc, the TMNP increased, suggesting that high levels selenium and zinc could compromise boar fertility. The increase in TMNP could be due to the elevated testosterone concentrations (Behne *et al*., 1996; Speigh *et al.*, 2010). There is evidence that increasing the dietary inclusion rate of selenium to 0.3 mg/kg improves reproductive performance of sows and boars (Close *et al.*, 2008). In the current study, both selenium and zinc supplementation influenced TMWP in both Large White × Landrace boars. At low levels of selenium and zinc, the TMWP increased but decreased at high level started, suggesting that high inclusion levels of selenium and zinc could be toxic to the boars. The increase in TMWP could be due to the reduction in the

inclusion levels of selenium and zinc in the diets. Dissanayake *et al.* (2012) reported that zinc incorporation improves sexual competence by increasing penile thrusting and prolonging ejaculatory latency without disturbing arousability.

The finding that the number of mounts was not influenced by inclusion levels of selenium and zinc in both LW × LR and Kolbroek boars suggests that libido levels may be be breed dependent (Rydhmer *et al.,* 2006). Kołodziej and Jacyno (2005) highlighted that increasing selenium supplementation from 0.2 to 0.5 mg/kg increased the number of mounts of boars Speight *et al.* (2012) reporetd increases in time mounting upon phantom, number of mounts and time of ejaculation in Landrace pigs supplementated with selenium. The increase in sexual behaviour score in pigs that were supplementated with zinc has also been reported in literature (Dissanayake *et al.,* 2009; Sabhapati *et al.,* 2016).

Semen volume was observed to be higher in LW × LR than Kolbroek boars. Masenya *et al.*, (2011) reported that Large White × Landrace boars had higher ejaculate volume than Kolbroek boars. Kondracki (2003) reported that indigenous pigs to have lower semen volume as compared to the standard semen volume 150 to 300 mL in improved European genotypes. Our findings showed that the ejaculation volume was not affected by selenium and zinc supplementation in Large White × Landrace and Kolbroek pigs. Increases in semen ejaculation volume in pigs supplemented with increasing levels of selenium has been reported earlier (Marin-Guzman *et al.,* 1997; Horky *et al.,* 2012). Lovercamp *et al.* (2013) reported that the increasing of dietary levels of selenium in the diet increased ejaculation volume of Yorkshire × Landrace × Large White pigs. Kołodziej and Jacyno (2005); Martins *et al.* (2018) reported that boars supplemented with selenium had high volume of ejaculate. Jacyno *et al.* (2002) reported that selenium supplementation increased ejaculate volume in boars while Horky *et al*.

(2011) reported that zinc supplementation increased the volume of ejaculate in breeding boars. Semen ejaculation volume increased as the levels of zinc supplementation increased (Zhao *et al.,* 2016). Selenium and zinc supplementation also increased semen ejaculate volume in Duroc boars (Horký *et al.* 2016b; 2016). However, studies on the interactions of selenium and zinc supplementation on semen volume have not been reported in pigs, but in turkeys (Ogbu *et al.* (2016). It is important to increases selenium and zinc on the diet because increases semen volume of both Kolbroek and LW x LR boars.

Testosterone concentration was not affected by selenium and zinc supplementation in both Large White × Landrace and Kolbroek pigs. Cheon *et al*. (2001) reported increases in testosterone concentrations in pigs that were supplemetated with selenium. El-Masry *et al.* (1994) and Meshreky *et al.* (2012) reported that testosterone concentration increased as levels of zinc supplementation increased in pigs. Borg (1993) reported that selenium supplementation increased testosterone concentrations in pigs. Kaya *et al.* (2006) and Imam *et al.* (2009) reported that testosterone levels increased as the levels of zinc supplementation increased in pigs. The higher testosterone concentrations in Large White × Landrace pigs than Kolbroek boars could reflect the influence of selection on male fertility. No genetic improvement programmes for Kolbroek pigs are not yet in place. Park and Yi. (2002) reported that testosterone concentrations were also higher in Yorkshire boars than in indigenous pigs. However, studies on interaction of selenium and zinc supplementation on testosterone concentration have focused on human (Neek *et al.* (2011) and cockerels (Abdalla *et al.,* 2015).

4.5 Conclusions

Selenium and zinc supplementation affect the levels testosterone, DE, sexual behaviour, number of mounts, TMWP and TMNP in pigs. There was no genotype × selenium on

testosterone concentration, ejaculation volume of both Large White × Landrace and Kolbroek boars. Thus, selenium and zinc supplementation of could be important for sexual reproduction and development of Large White × Landrace and Kolbroek boars.

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Chapter 5

Interaction of selenium and zinc supplementation on visceral weights and carcass traits of Large White × Landrace and Kolbroek boars

Abstract

The objective of the current study was to determine the interactions among genotypes, selenium and zinc supplementation on visceral weights and carcass characteristics of Large White × Landrace (LW × LR) and Kolbroek boars. The boars were assigned to four experimental groups in a 2 ×2 × 2 factorial arrangement in a completely randomized block design with six boars per treatment were used. The pigs between 7 to 8 months old at the average with 81.6 ± 1.7 kg average body weight of LW × LR (n = 24) and Kolbroek (n = 24) with average body weight 74.2 ± 0.6 kg were used for study. Forty-eight boars of each genotype were fed on one of four treatment combinations of high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) and low selenium (0.26 mg/kg) and low zinc (0.35 mg/kg) namely: high selenium high zinc (HSHZ), high selenium low zinc (HSLZ), low selenium high zinc (LSHZ) and low selenium low zinc (LSLZ). The pigs were kept for six months. At slaughter, carcass traits were determined. Backfat thickness was taken at (first ribdorsal fat thickness (DFT1 last rib (dorsal fat thickness at last rib (DFT2)) and and last lumbar vertebra (dorsal fat thickness at last lumbar vertebra (DFT3). The rib weight (RW), hindquarter weight (HQW), hindquarter length (HQL) and the hindquarter circumference (HQC), warm carcass weight (WCW), cold carcass weight (CCW), rib weight proportion (RWP), shoulder weight proportion (SWP) and hindquarter weight proportion (HQWP) were determined. Selenium and zinc supplementation had not effect on visceral organ weight, carcass traits and primal pork cuts in both genotypes ($P > 0.05$). There were genotype differences in the weights of large and small intestines, WCW, CCW, CL weight and DL, HQL, HQC, DFT1, DFT2 and DFT3 ($P < 0.05$). In conclusion, selenium and zinc

supplementation had no effect on carcass traits and primal pork in LW × LR pigs. These findings suggest that Se and Zn while have a greater role physiological role, they might no and or mimimal role on visceral organ and meat output.

Keywords*:* crossbreed, indigenous pigs, carcass, minerals

5.1 Introduction

Selenium absorption rates in pigs lie between 75 and 85 % at the level of the basal requirement 0.3 mg/kg (Luginbuhl, 1998). Selenium has higher absorption and biological effectiveness in pigs than in goats, cattle and sheep (Jang *et al.* 2010; Mahan *et al.*, 2014), broilers (Mikulski *et al.*, 2009; Briens *et al.*, 2013), and turkeys (Mahan *et al.*, 1999; Juniper *et al*., 2011). Downs *et al*. (2000) observed improved pork quality and oxidative stability from Large White pigs fed on increased levels of selenium. Selenium plays an important role in immune function and production of immune globulins (Rao *et al.*, 2013), Selenium supplementation has been reported to improve the nutritional value and quality characteristics of pork products (Surai, 2006). Little information is known to assessed the effect of combining selenium and zinc on the carcass traits and meat quality in LW × LR and Kolbroek pigs. Selenium has also been considered in reducing meat spoilage.

The consumer demand for lean meat is on the increase (Peres *et al.*, 2014). The challenge in the pig industry is to improve the nutritional value, quality, and shelf life of pork. Selenium supplementation might improve carcass characteristics such warm and cold carcass, dressing percentage and water holding capacity (Oliveira *et al.*, 2014) and, in addition, maintain a healthy immune system of both the pigs and consumers. Selenium supplementation may also enrich the mineral content of the pork, making it healthier for the consumers (Grashorn, 2007).

The importance of selenium and zinc supplementation on meat traits in Kolbroek pigs, largely is scant.

Edmonds and Arentson (2001) reported that pigs fed on diets with low levels of selenium and zinc had reduced carcass performance. There is limited information on the influence of selenium and zinc supplementation on warm, and cold carcass, dressing percentage and water holding capacity of LW × LR and Kolbroek pigs. There is currently limited research on effects of selenium and zinc on carcass traits and visceral organs in the LW × LR and Kolbroek pigs. Most of the previous studies (e.g. Mahan and Parrett, 1996; Mahan *et al.,* 1999) focussed on different sources of selenium on pis carcass performance. The carcass classification systems, including South Africa's PORCUS system are largely based on subcutaneous fat thickness and, therefore, the main focus is placed on increasing the lean: fat ratio of the carcass (Needham and Hoffman, 2015). Selenium supplementation reduced drip loss and improved muscle colour in pigs (Juniper *et al.,* 2008; 2011; Mateo *et al.,* 2007; Calvo *et al.,* 2017). Pale, soft, exudative meat, (PSE) describes a carcass quality condition known to occur in pork, beef, and poultry. It is characterized by an abnormal colour, consistency, and water holding capacity, making the meat dry and unattractive to consumers. Selenium utilization and its effects on meat quality and muscle characteristics since PSE fillets from chickens present a compromised enzymatic antioxidant defense system with lower GSH-PX activity (Dos Santos *et al.* 2012). However, there is no information available on how selenium and zinc interaction on carcass carcass characteristics of Large White × Landrace and Kolbroek pigs.

The absorbed selenium, which is not immediately metabolized, is incorporated into certain tissues with high levels of protein synthesis, such as the pancreas, liver, kidney and stomach, and gastrointestinal epithelium (Oliveira *et al.,* 2014). Hence the current study sought to assess

the effect of selenium and zinc supplementation on visceral organs, carcass traits and primary pork of LW × LR and Kolbroek boars. It was hypothesized that there is interaction of selenium and zinc on visceral organs, drip loss and meat quality of LW × LR and Kolbroek pigs.

5.2 Materials and methods

4.2.1 Description of study site

The study was conducted at the Pig Research Unit of Agricultural Research Council-Animal Production (Germplasm Conservation & Reproductive Biotechnologies Unit), Irene, South Africa. The Agricultural Research Council, Animal Production Institute campus is located at 25° 55′ South; 28° 12′ East and is located in the Highveld Pretoria of Republic of South Africa and situated at an altitude of 1525m above sea level. Experimental boars were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (Ref: APIEC16/037) during the period from November 2016 to February 2017.

5.2.2 Experimental pigs and housing

Large White × Landrace and Kolbroek boars were assigned to four experimental groups in 2 × 2 × 2 randomised factorial arrangements with sixboars per treatment. Forty-eight boars of 7 to 8-months of age and with (81.6 ± 1.7 kg) average body weight of LW × LR (n = 24) and Kolbroek (n = 24) with average body weight (74.2 ± 0.6 kg) were used in the study. The boars were allowed an adaptation period of two weeks to the experimental diets and received water *ad libitum*. Each boar received 2 kg per day until the end of the experiment. The boars were fed for six months. The pens were cleaned daily and the pigs were dipped in Triatix® pig pour-on (Amitraz) every two weeks against ectoparasites and were dewormed once a month against

endo-parasites using Valbazen® (Albendazole). Each boar was housed in 2 × 1.5 m pens in environmentally controlled house with the temperature ranging from 22 to 25°C.

5.2.3 Experimental diets and feeding

Six pigs of each genotype were randomly assigned to four experimental diets containing either high or low levels of selenium and zinc. The four diets were: low selenium (0.26 mg/kg) and low zinc (0.35 mg/kg) (LSLZ); high selenium (0.65 mg/kg) and high zinc (0.74 mg/kg) (HSHZ); low selenium (0.26 mg/kg) and high zinc (0.74 mg/kg) (LSHZ); and high selenium (0.65 mg/kg) and low zinc (0.35 mg/kg) (HSLZ). Ingredient and chemical composition of the diets are shown in Table 5.1.

A total mixed ration was formulated to supply14 MJ/kg digestible energy, 180 g crude protein (CP)/kg DM and 11.6 g lysine/kg which meet the requirements of growing pigs (NRC, 1998). The dry matter (DM), ash, crude protein (CP), ether extract (EE), zinc, selenium, and acid detergent fibre (ADF) were analysed following the procedures of the Association of Official Analytical Chemists (2005) and neutral detergent fibre (NDF) was determined following the procedures of van Soest (1963), the ingredient and chemical composition of the diets.

Table 5.1: Ingredient and chemical composition of the diets

Ingredient (%)	Diets[1]			
	HSLZ	HSHZ	LSHZ	LSLZ
Yellow maize	62.25	62.25	62.25	62.25
Hominy chop	8.64	8.64	8.64	8.64
Feed lime	0.27	0.27	0.27	0.27
Monocalcium phosphate	2.95	2.95	2.95	2.95
Soya bean oil cake	24.69	24.69	24.69	24.69
Vitamin mineral premix[3]	0.20	0.20	0.20	0.20
Salt	1.00	1.00	1.00	1.00
Zinc	0.35	0.74	0.74	0.35
Selenium	0.65	0.65	0.26	0.26
Chemical composition analysis (%)				
Protein	16.74	16.74	16.17	16.72
Dry matter	90.45	90.65	90.28	90.22
Crude fibre	3.31	3.08	3.31	3.59
NDF	20.17	21.47	19.95	19.59
ADF	4.58	4.94	4.41	4.96
Digestible energy MJ/kg	20.31	20.71	20.44	20.21
Phosphorus	0.87	0.72	0.78	0.89
Calcium	0.80	0.60	0.76	0.68
Zinc	0.04	0.06	0.06	0.04
Selenium	0.006	0.006	0.001	0.001

[1]HSLZ = high selenium, low zinc; HSHZ = high selenium, high zinc; LSHZ = low selenium, high zinc and LSLZ = low selenium, low zinc

[2]NDF = neutral detergent fibre, ADF = acid detergent fibre

[3]The following minerals;4.5 mg vitamin B2, 0.03 mg vitamin B12, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous.

5.2.4 Carcass measurements

All boars were slaughtered after six months of feeding on the respective experimental diets and processed according to the routine abattoir procedures. Briefly, the pigs were then stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 s and exsanguinated within 10 s of stunning. Warm carcass weight (WCW) was measured after dressing using an over head scale. Visceral organs were removed immediately after removing the remaining hair. The hair was removed with a gas naked flame. Automatic weighing scale was used to measure the weight of the pigs. After the removal of the visceral organs, the carcass weight then determined and expressed as percentage of live weight to get the dressing percentages. The carcass was put in cold room for 24 hours after which the cold carcass weights (CCW) and length of carcass (CL) for each pig were determined. The carcass length was taken as a distance from anterior edge of the first rib to the pubic bone along median plane using a measuring tape (Kanengoni *et al.*, 2014). Each carcass was then cut at the last rib up to the middle. All other carcass measurements were taken from the left side.

A cut was made between the l0th and 11th ribs and carried on through the spinal column. The fat measurement was taken on each carcass with Vernier callipers over the eye muscle, 60 mm from the carcass midline. Thickness of the backfat (BFT) was measured using a pair of Vernier callipers (Future Light (Gauteng, 0.05 mm). Backfat measurements were taken at first rib (DFT1), last rib (DFT2), and last lumbar vertebra (DFT3). Ham was separated by locating the division between the 2^{nd} and 3^{rd} sacral vertebrae and sawn perpendicularly along the axis of the ham. The shoulder was removed by cutting between the third and fourth ribs caudally and junction of the caudal edge of the second rib with the sternum cranially, with fronttrotter removed by cutting through the metacarpal region.

5.2.5 Primal pork cuts measurements

The rib was weighed to obtain the rib weight (RW). The hind leg was removed between the second and third sacral vertebrae perpendicular to the stretched leg and at the hock joint distally and weighed to get the hindquarter weight (HQW). The hind leg was measured to get the hindquarter length (HQL), from the ischiopubic symphysis to the hock joint and the hindquarter circumference (HQC) in the area of maximum amplitude near the base of the tail. The RW, SW and HQW were measured as a proportion of CCW to give RWP, SWP and HQWP, respectively.

5.2.6 Statistical analyses

The genotype × selenium × zinc interaction on carcass traits were performed using the GLM procedure version 9.3 statistical software of SAS, 1999. A 5% significance level was used.

The model used was:

$Y_{ijkl} = \mu + S_i + Z_j + G_k + (S \times Z)_{ij} + (S \times G)_{ik} + (Z \times G)_{jk} + (S \times Z \times G)_{ijk} + E_{ijkl}$

Y_{ijkl} = Visceral morphology and carcass traits

μ = is the overall mean common to all observations

S_i = Se level (i = LS, HS)

Z_j = Zinc level (j = LZ, HZ)

G_k =Genotype (k = Kolbroek, Large White × Landrace)

$(S \times Z)_{ij}$=Se× zinc interaction

$(S \times G)_{ik}$ =the interaction of selenium × genotype

$(Z \times G)_{jk}$= the interaction of zinc × genotype

$(S \times Z \times G)_{ijk}$= the interaction of selenium × zinc × genotype

E_{ijkl}= the residual error.

5.3 Results

5.3.1 Visceral organs

The visceral organ weights as proportions of final body weights of Large White × Landrace and Kolbroek boars fed on different dietary levels of selenium and zinc are shown in Table 5.2. There was no effect of genotype × selenium × zinc, selenium × zinc, genotype × selenium and genotype × zinc interactions on the pancreas, stomach, liver, lungs and heart indices ($P > 0.05$). There was no effect of genotype, selenium and zinc had on pancreas, stomach, liver, lungs and heart indices of both Large White × Landrace and Kolbroek boars ($P > 0.05$). There were genotype × selenium interactions on the large and small intestines ($P < 0.05$). Large White × Landrace pigs fed on the HSHZ diet had large and small intestine indicesthan those fed on the LSLZ diet, while Kolbroek boars fed on the HSHZ diet had lower large and small intestine indices than those fed on the LSHZ diet ($P < 0.05$).

Table 5.2: Effects of selenium and zinc supplementation on the visceral organ indices of Large White × Landrace (n = 24) and Kolbroek (n = 24) pigs

Inclusion levels, mg/kg			Visceral organs indices, g/kg						
Genotypes	Selenium	Zinc	Pancreas	Stomach	Liver	Lungs	Heart	Large intestine	Small intestine
LW × LR	High	High	0.25	1.54^{ab}	0.82^{b}	1.09	0.28	3.87^{a}	3.65^{a}
	High	Low	0.26	1.52^{ab}	1.01^{a}	1.07	0.28	3.57^{abc}	3.32^{ab}
	Low	High	0.25	1.54^{ab}	0.90^{ab}	1.10	0.28	3.36^{abcd}	3.10^{abc}
	Low	Low	0.25	1.49^{b}	0.86^{ab}	1.23	0.26	2.92^{cd}	2.71^{bc}
Kolbroek	High	High	0.28	1.57^{ab}	1.04^{ab}	1.07	0.27	2.81^{d}	2.56^{c}
	High	Low	0.29	1.49^{b}	1.03^{ab}	1.09	0.29	3.26^{abcd}	3.06^{abc}
	Low	High	0.29	1.53^{ab}	0.82^{b}	1.06	0.27	3.71^{ab}	3.52^{a}
	Low	Low	0.26	1.58^{a}	0.98^{ab}	1.07	0.27	3.06^{bcd}	2.73^{bc}
SEM			0.2518	0.3609	0.2624	0.0876	0.8920	0.2033	0.1609
P value	Genotype		NS	NS	NS	NS	NS	***	**
	Selenium		NS	NS	NS	NS	NS	NS	NS
	Zinc		NS	NS	NS	NS	NS	NS	NS
	Genotype × selenium		NS	NS	NS	NS	NS	**	**
	Genotype × zinc		NS	NS	NS	NS	NS	NS	NS
	Selenium × zinc		NS	NS	NS	NS	NS	NS	NS
	Genotype × selenium × zinc		NS	NS	NS	NS	NS	NS	NS

[ab] Values with different letters in a column differ significantly *** P < 0.001, ** P < 0.01, * P < 0.05 and NS = not significant, LW × LR = Large White × Landrace, SEM = standard error mean Low selenium low zinc (HSLZ) (0.65, 0.35 mg/kg), high selenium high zinc (HSHZ) (0.65, 0.74 mg/kg), low selenium high zinc (LSHZ) (0.26, 0.74 mg/kg) and low selenium low zinc (LSLZ) (0.26, 0.35 mg/kg)

5.3.2 Carcass characteristics

The carcass traits of Large White × Landrace and Kolbroek boars fed diets containing selenium and zinc supplementation are shown in Table 5.3. There were genotype × selenium × zinc interactions on warm carcass weights ($P < 0.05$) as shown in Table 5.3 and Figure 5.1. The warm carcass weights in Large White × Landrace pigs fed on the low zinc diet were similar ($P <0.05$) irrespective of selenium levels (high vs low) while Kolbroek pigs fed on a high selenium diet had a lower warm carcass weight ($P < 0.05$) when zinc was low compared to high zinc concentrations. There was no genotype × zinc × selenium, selenium × zinc, genotype × zinc and genotype × selenium interactions on cold carcass weight (CCW), dressing percent (DP), drip loss (DL) and carcass length (CL) ($P > 0.05$). There were however genotype effects ($P < 0.05$) on WCW, CCW, DL and CL with LW × LR having higher values than in the Kolbroek. There were no selenium and zinc effect on warm carcass weight and dressing percentage of both genotypes ($P>0.05$).

Table 5.3: Carcass traits of Large White × Landrace and Kolbroek boars fed on selenium and zinc supplements

Genotype	Diets[2]		Parameters[1]				
	Selenium, mg/kg	Zinc mg/kg	WCW	CCW	DP	DL	CL
Kolbroek	High	High	58.0[b]	57.1[b]	71.2	2.7[a]	64.2[a]
	High	Low	58.4[b]	57.2[b]	71.3	2.8[a]	64.2[a]
	Low	High	58.3[b]	57.0[b]	71.1	2.7[a]	64.1[a]
	Low	Low	58.2[b]	57.2[b]	71.1	2.7[a]	64.4[a]
	SEM						
LW×LR	High	High	68.1[a]	67.1[a]	71.3	3.4[b]	67.3[b]
	High	Low	68.1[a]	67.2[a]	71.3	3.3[b]	67.1[b]
	Low	High	67.9[a]	67.0[a]	71.3	3.3[b]	67.2[b]
	Low	Low	68.1[a]	67.0[a]	71.2	3.2[b]	67.3[b]
SEM			0.08	0.11	0.09	0.06	0.17
P value	Genotype		***	***	NS	***	***
	Se		NS	NS	NS	NS	NS
	Zn		*	NS	NS	NS	NS
	Genotype × Se		NS	NS	NS	NS	NS
	Genotype × Zn		NS	NS	NS	NS	NS
	Se × Zn		NS	NS	NS	NS	NS
	Genotype × Se × Zn		**	NS	NS	NS	NS

[a,b]within a column means with different superscripts differ (P < 0.05), *** P < 0.001, ** P < 0.01, * P < 0.05 and NS = not significant, LW × LR = Large White × Landrace

[1]WCW = warm carcass weights; CCW = cold carcass weights (kg); DP = dressing percentage; CL - carcass length (cm); DL = drip loss (%),

[2]Low selenium low zinc (HSLZ) (0.65, 0.35 mg/kg), high selenium high zinc (HSHZ) (0.65, 0.74 mg/kg), low selenium high zinc (LSHZ) (0.26, 0.74 mg/kg) and low selenium low zinc (LSLZ) (0.26, 0.35 mg/kg)

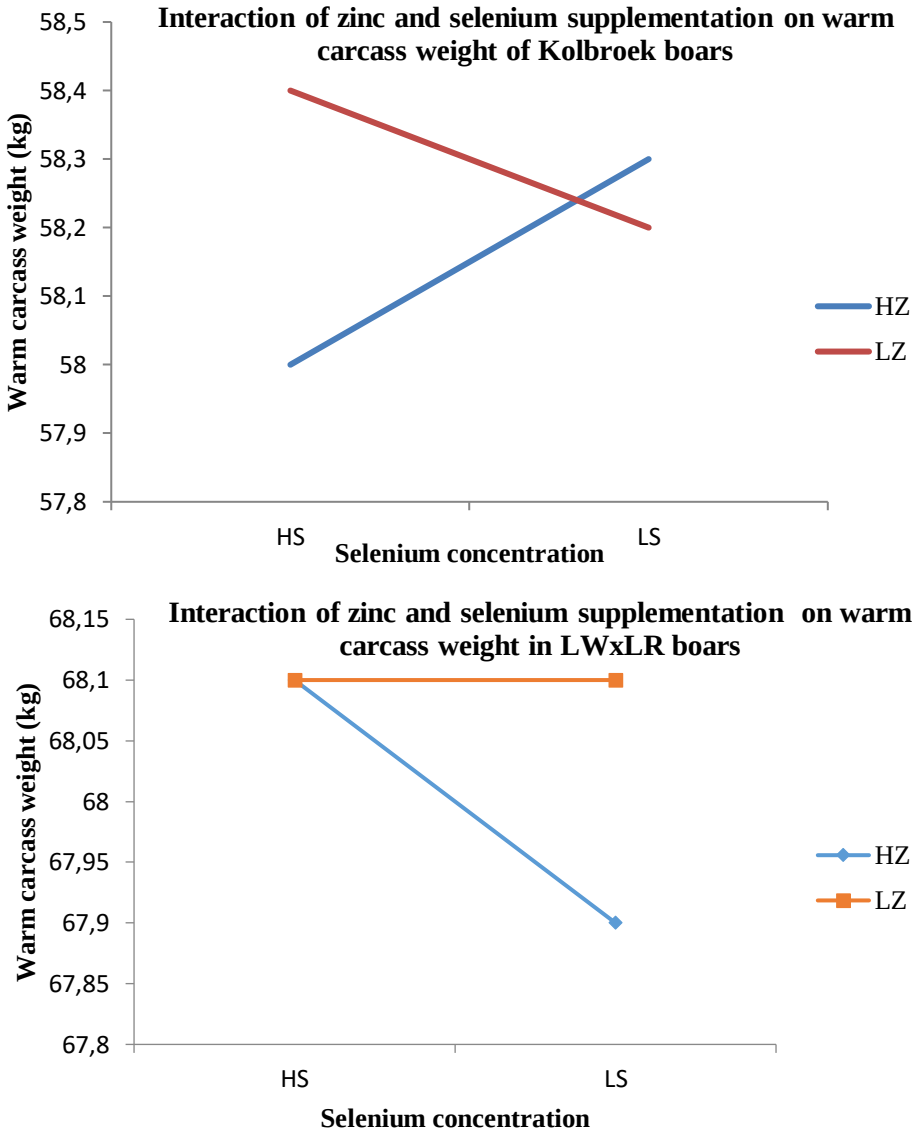

Figure 5.1: Interaction of zinc and selenium supplementation on warm carcass weights in Kolbroek and Large White × Landrace boars (HS – high selenium, LS – low selenium, HZ – high zinc, LZ – low zinc)

5.3.3 Primal pork cuts measurements

The primal pork cuts measurements in Large White × Landrace and Kolbroek pigs and pigs fed diets containing selenium and zinc are given in Table 5.4. There were genotype x selenium interactions in hindquarter lengths (HQL) ($P < 0.05$). There were no effects of zinc and selenium on hindquarter lengths in the Kolbroek ($P > 0.05$). However, in the LW x LR, the hindquarter lengths were similar ($P <0.05$) and lower at low selenium concentrations irrespective of zinc concentrations but higher at high selenium concentrations ($P < 0.05$) as shown in Figure 4.2. There were selenium × zinc interactions for hindquarter circumference (HQC) ($P < 0.05$). The hindquarter circumferences were similar ($P <0.05$) at low selenium concentrations irrespective of zinc concentrations in the Kolbroek boars but higher at high selenium concentrations ($P < 0.05$) as shown in Figure 4.2. In the LW × LR on the other hand, the hindquarter circumference of pigs fed on low selenium and low zinc was lower than on high selenium and low zinc, while pigs fed on high zinc and high selenium is lower than those fed low selenium and high zinc ($P < 0.05$). The dorsal fat thickness in Kolbroek were similar ($P< 0.05$) and lower at low selenium concentrations, irrespective of zinc concentrations but higher at high selenium concentrations ($P < 0.05$) as shown in Figure 5.2. There were genotype effects ($P < 0.05$) for dorsal fat thickness 2 (DFT2) and dorsal thickness 3 (DFT3).

Table 5.4: Effect of dietary selenium and zinc on primal pork cuts measurements in Large White × Landrace n = 24 and Kolbroek n = 24 pigs

Genotype	Diets[3]		Parameters[2]							
	Selenium	Zinc	HQL	HQC	HQWP	RWP	SWP	DFT1	DFT2	DFT3
Kolbroek	High	High	29.2[a]	46.1[a]	27.0	13.1	11.5	36.3[a]	23.0[a]	22.2[a]
	High	Low	29.2[a]	46.3[a]	27.2	13.2	11.1	36.1[a]	22.9[a]	22.2[a]
	Low	High	29.2[a]	46.0[a]	27.2	13.1	11.4	36.0[a]	23.1[a]	22.0[a]
	Low	Low	29.2[a]	46.0[a]	27.1	13.1	11.2	36.0[a]	23.1[a]	22.0[a]
LW x LR	High	High	35.3[b]	52.1[b]	26.9	13.3	11.0	28.4[c]	17.1[b]	16.0[b]
	High	Low	35.2[b]	52.3[b]	27.1	12.8	11.1	28.2[bc]	16.9[b]	15.9[b]
	Low	High	35.0[b]	52.2[b]	27.1	13.1	11.9	28.1[bc]	17.1[b]	16.0[b]
	Low	Low	35.0[b]	52.0[b]	27.0	13.1	11.0	27.9[b]	17.1[b]	16.0[b]
SEM			0.07	0.10	0.20	0.16	0.33	0.09	0.11	0.08
P value	Genotype		***	***	NS	NS	NS	***	***	***
	Se		*	NS	NS	NS	NS	**	NS	0.07
	Zn		NS	NS	NS	NS	NS	*	NS	0.08
	Genotype× Se		*	NS	NS	NS	NS	NS	NS	NS
	Genotype × Zn		NS	NS	NS	NS	NS	NS	NS	NS
	Se × Zn		NS	*	NS	NS	NS	NS	NS	NS
	Genotype × Se × Zn		NS	NS	NS	NS	NS	NS	NS	NS

[a,b] Means with different letters in a column differ significantly (P < 0.05), (P < 0.05), *** (P < 0.001), ** (P < 0.01), * (P < 0.05) and NS = not significant,
[2]HQL = hind quarter length (cm), SWP = shoulder weight proportion (%), RWP = rib weight proportion (%), DFT1 = dorsal fat thickness at first rib (mm); DFT2 = dorsal fat thickness at last rib (mm); DFT3 = dorsal fat thickness at last lumbar vertebra (mm)
[3]Low selenium low zinc (HSLZ) (0.65, 0.35 mg/kg), high selenium high zinc (HSHZ) (0.65, 0.74 mg/kg), low selenium high zinc (LSHZ) (0.26, 0.74 mg/kg) and low selenium low zinc (LSLZ) 0.26, 0.35 mg/kg [1]LW × LR= Large White × Landrace, n = 6 per diet and KB = Kolbroek, n = 6 per diet

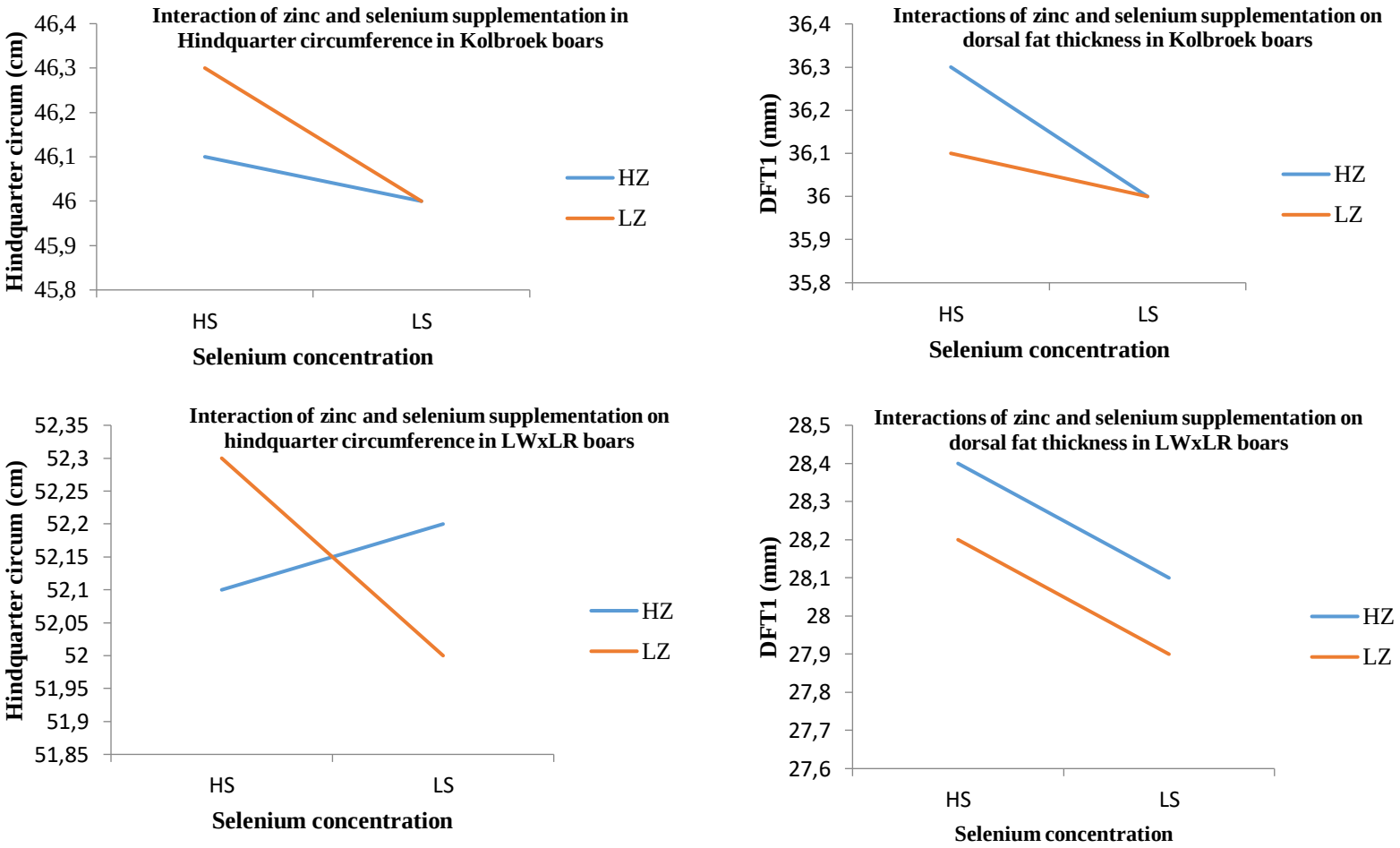

Figure 5.2: Interactions of zinc and selenium supplementation on hindquarter circumference and dorsal fat thickness (DFT1) in LW x LR and Kolbroek boars

5.4 Discussion

The finding that selenium and zinc supplementation did not influence WCW of LW × LR and Kolbroek was not expected. There was influence of genotypes on WCW of both The Large White × Landrace and Kolbroek pigs. The Large White × Landrace pigs had higher WCW weight than Kolbroek pigs. The Large White × Landrace ability to consume more feed per metabolic weight than the Kolbroek pigs. The Large White × Landrace pigs produced commercial warm carcass weights that were 0.4 kg lighter than Kolbroek pigs when adjusted to a constant body weight, most of which could be accounted for by differences in full gut weight. The LW × LR pigs were expected to consume more feed than the Kolbroek pig because of their bigger body size and gut capacity (Whittemore *et al*., 2003). Wolter *et al.* (1999) reported that increasing inclusion levels of selenium supplementation, the WCW was increasedin growing–finishing pigs. Mateo *et al.* (2007) reported that as dietary selenium concentration increased, the WCW increased in Camborough 22 × PIC boars. Svoboda *et al.* (2009) reported that as dietary selenium supplementation increased, the WCW of Landrace × Czech Large White pigs also increased. A similar pattern was observed in Yorkshire × Landrace boars (Speight *et al.*, 2012). Hernandez *et al.* (2008) reported that there was increased of zinc supplementation with the increased of WCW in Large White × Landrace pigs. The warm carcass weight increased as zinc level increased of TR4 (Fast × L02 PIC) pigs (Carpenter *et al.*, 2016). Increasing zinc in diets increased WCW in growing pigs (Paulk *et al.*, 2015).

The finding that inclusion levels of selenium and zinc did not influence cold carcass weight (CCW) of LW × LR and Kolbroek pigs was also consistent with WCW findings. The CCW was affected by genotype. The Large White × Landrace pigs had higher CCW weight than Kolbroek pigs, was expected. The dietary levels of selenium increased with the increased in CCW in Yorkshire × Landrace boars (Speight *et al*., 2012). There was an increase in CCW

with increased the inclusion levels of selenium (Wolter *et al*., 1999). Saikia *et al.* (2016) reported increases in CCW in Hampshire × Assam local pigs supplemented with zinc. The CCW increased as inclusion levels of zinc supplementation increased in Duroc × Landrace × Yorkshire (Xu *et al.*, 2017). However, studies on interaction of selenium and zinc supplementation on CCW have focused on broiler chickens (Hong-mei *et al.*, 2011).

The dressing percentage (DP) was not affected by selenium and zinc supplementation in LW x LR and Kolbroek pigs. The DP was not affected by genotype. Wolter *et al.* (1999) reported increases in DP in finishing pigs supplemented with selenium. Dressing percentage increased as levels of selenium increased in Yorkshire x Landrace boars (Speight *et al.*, 2012). Saikia *et al.* (2016) reported that zinc supplementation lead to increases in DP in Hampshire × Assam local pigs. Hernandez *et al.* (2008) reported increases DP in Large White × Landrace pigs supplemented with zinc. However, studies on selenium × zinc interaction on DP have focused mostly on chickens (Xu *et al.*, 2003).

The inclusion levels of selenium and zinc did not affect drip loss (DL) of LW × LR and Kolbroek boars. There was an effect of genotype on DL of LW × LR and Kolbroek boars. Nevrkla and Václavková (2018) reported that Prestice Black-Pied pigs reached lower drip loss values for Large White × Landrace × Duroc × Pietrain pigs, indicating that meat of native breeds could be characterized by slower pH decrease post-mortem. Li *et al*. (2011) noticed that the higher the selenium supplementation, the lower the DL. Zhan *et al.* (2007) reported that supplementation of selenium at the amount of 0.045 mg/kg of feed increased meat colour parameters an and also lowered the DL value. Mahan *et al.* (1999) reported that a higher DL in the meat of fatteners fed with inorganic selenium additive. Drip loss percentages increased as levels of selenium supplement increased in Camborough 22 pigs Mateo *et al.* (2007). The DL

increased as added dietary selenium concentration increases in pigs fed diets containing selenium supplementation in pigs (Mateo *et al*., 2007). Boleman *et al.* (1995) reported no effect on DL increased, with the increasing the inclusion levels of selenium in the pig diet. However, studies on interaction of selenium and zinc supplementation on DL have focused on broiler chickens (Hong-mei *et al.,* 2011).

The small intestine, lung, heart, liver and stomach weights were not affected by selenium and zinc supplementation in LW × LR and Kolbroek pigs. There was, however, an effect of genotype on small and large intestine weights. Literature have cited increased weight of heart, lung and the liver, small intestine in growing pigs with selenium supplementation (O'Dell and Sunde, 1997; Tian *et al.,* 2006; Mehdi *et al*., 2013; Jlali *et al*. (2014). Mateo *et al.* (2007) reported increases in liver, heart, lungs and intestine in Camborough 22 pigs supplementated with selenium. Calvo *et al.* (2016) reported increases in liver, heart, lungs in pigs supplementaed with increasing levels of zinc. The small and large intestine increased as levels of zinc supplementation increased in pigs (Li *et al*., 2001). However, studies on interaction of selenium and zinc supplementation on intestine, liver, pancreas, kidneys, spleen, liver, and lungs organs indices have focused on rats (Chmielnicka *et al.,* 1988) and liver in fish (Tarassoli *et al.,* 2012). Such information is, however, scarce in LW × LR and Kolbroek pigs.

The finding that the HQL was not influenced by selenium and zinc inclusion levels in the diet indicates that selenium and zinc was utilized to maintain the carcass traits. Large White × Landrace had higher HQL than Kolbroek pigs, as expected. Large White × Landrace pigs, increase the inclusion levels of selenium and zinc with increased HQL. The increase in cut percentages seems to be largely due to increased lean muscle growth rather than decreased fat deposition, with the exception of the hindquarter (Needham and Hoffmanm, 2015). However,

indigenous genotypes of southern Africa, do not contribute greatly to commercial pork production, because they have smaller carcasses and tend to deposit fat early when fed high-energy diets (Madzimure *et al*., 2017). The finding that the Kolbroek pigs had a higher DFT1, DFT2 and DFT3 than LW × LR pigs, was, therefore, expected. Feed conversion into muscle in Kolbroek pigs is low since most of the dietary nutrients are converted into body fat. The Kolbroek pigs had higher RWP than LW × LR pigs. At high level of selenium and zinc, the RWP increase and low level started decreasing, suggesting that the high levels selenium and zinc could create acidic conditions. Because of the fat deposition pattern in pigs, backfat thicknesses are generally thinner at the last rib than at the more anterior region of the animal's back (Tess *et al*., 1986). The observation that genotype × selenium × zinc no effect on primal pork cuts measurements. Selenium and zinc supplementation had no influence on primal pork cuts measurements. Madzimure *et al*. (2012) reported that Windsnyer had a thicker subcutaneous fat layer than the Large White pigs. The back fat thickness increased with an increase in zinc supplementation (Saikia *et al.*, 2016). Increases in fat meat percentage, lean eye area, and backfat thickness in pigs supplementated with increasing levels of dietary zinc have been reported (Wolter *et al.*, 1999; Zhang *et al*., 2014). Selenium was administered during the whole course of the fattening period and showed a marked increase in backfat thickness as opposed to the group without any selenium addition (Čítek *et al.*, 2012). However, pigs fed the diets containing the selenium-enriched yeast produced carcasses with lower backfat depths at the last lumbar vertebra and larger loin-eye areas compared with animals consuming the inorganic source of dietary selenium (Wolter *et al.*, 1999). Information on how does interaction of selenium × zinc influence the weight of primal pork cuts measurements of Kolbroek pigs needs to be provided.

5.5 Conclusions

The present study provides evidence for the positive effect of selenium and zinc supplementation on weight of small and large intestine of LW × LR and Kolbroek pigs. Increasing the levels of both selenium increased the weight and length of carcass traits and primal pork in LW × LR and Kolbroek pigs. Supplementation of selenium and zinc did not increase the carcass traits and primal porkof both genotypes.

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Chapter 6

Selenium × zinc interaction on epididymis spermatozoa quality, seminal plasma constituents and lipid peroxidation of Large White × Landrace and Kolbroek boars

Abstract

The objective of the current study was to examine the effect of different dietary levels of selenium and zinc interactions on epididymal spermatozoa quality, seminal plasma (SP) and lipid of Large White × Landrace and Kolbroek boars. Forty-eight Large White × Landrace boars (n=24) with average body weight (BW) of 81.6 ± 1.7 kg and Kolbroek boars (n=24) with average BW of 74.2 ± 0.6 kg between 7 to 8 months of age were used in this study. Boars were selected and fed for six months on one of four diets in a 2 × 2 × 2 (genotype × zinc concentration × selenium concentration) factorial design. Selenium levels were supplied at either low (0.26 mg/kg) or high (0.65 mg/kg) concentrations. Zinc levels were supplied at low (0.35 mg/kg) while 0.74 mg/kg was defined as high. Spermatozoa motility was deternined using a computer-aided sperm analysis (CASA). Spermatozoa viability was assessed using hyper osmotic swelling test (HOST) test, while lipid peroxidation was determined using the malondialdehyd (MDA) test. Seminal plasma constituents were assessed using atomic absorption spectrophotometriy. There were no effects of genotype on macroscopic semen evaluation ($P > 0.05$). There were no effects of selenium and selenium supplementation on semen volume and spermatozoa concentration of both genotypes ($P > 0.05$). The spermatozoa abnormalities were influenced by selenium and zinc supplementation ($P < 0.05$), except tail abnormalities ($P > 0.05$). Selenium and zinc supplementation also influenced, VCL and amplitude of lateral head (AH) ($P < 0.05$). There were also effects of selenium and zinc supplementation on progressive motility (PM), non-progressive motility (NPM) of both genotypes ($P<0.05$). The seminal plasma constituents of zinc, potassium and calcium were not influenced by selenium and zinc

supplementation (P > 0.05). However, sodium, selenium plasma constituents, and (P) (P <0.05) were affected by inclusion levels of selenium and zinc of both genotypes. Lipid peroxidation MDA levels were affected by the dietary levels of selenium and zinc (P<0.05). In conclusion, increasing the levels of selenium and zinc increased quality of spermatozoa, zinc, phosphours and calcium of both genotypes.

Keywords: Kolbroek, minerals, motility, MDA; biochemical constituents

6.1 Introduction

The Kolbroek pig is an early maturing genotype, reaching puberty at four to five months. However, these pigs grow slowly than imported pig genotypes (Chimonyo *et al.*, 2005). There is concern about the small genetic variation within the Kolbroek pig genotype and a danger of the genotype becoming extinct because of indiscriminate crossbreeding (Chimonyo *et al*., 2005). The Kolbroek boars may potentially contribute to crossbreeding for improving adaptability traits of pigs (Mapeka *et al.,* 2009). The reproductive potential of the Kolbroek boars has, however, not been fully exploited compared to other pig genotypes. Masenya *et al.* (2013) indicated that there is lack of accurate methods of predicting the fertility rate of Kolbroek boar spermatozoa.

Little information is known on the effects of different dietary inclusion levels of selenium and zinc on epididymis spermatozoa quality, biochemical proteins in seminal plasma (SP) constituents and lipid peroxidation of LW × LR and Kolbroek pigs. Both zinc and selenium playing a huge role in spermatozoa production and antioxidant status in sheep (Kendall *et al.*, 2000). Selenium and zinc are important for normal reproductive function in boars (Villaverde *et al*., 2014). Therefore, selenium and zinc supplementation could be necessary for

spermatozoa traits of LW × LR and Kolbroek pigs (Wong *et al.*, 2001). There is, however scarce literature on the effects of boar genotypes, selenium and zinc on the seminal plasma biochemical variables (Zaja *et al.*, 2016).

The ability of spermatozoa to fertilize ova depends on the lipid composition in spermatozoa and seminal plasma (Chatiza *et al.*, 2018). The semen, seminal plasma and spermatozoa influence boar characteristics. The use of seminal plasma to decrease the amount of damage that occurs during the process of cryopreservation requires further understanding. (Zaja *et al.*, 2016). Seminal plasma is comprised of ions, organic and nitrogenous substances, among other compounds (Villaverde *et al.*, 2014). Aside from its function in spermatozoa maturation, metabolism and function in the boar, Seminal plasma is essential for the transportation and survival of spermatozoa in the female reproductive tract. Boar spermatozoa are sensitive to lipid peroxidation due to the high content unsaturated fatty acids in the phospholipids of their plasma membranes and relatively low antioxidant capacity (Amin *et al.*, 2010).

Zinc is important for the attachment of head to tail of spermatozoa and is required for the production of an antibacterial compound released from the prostate gland into the semen (Kendall *et al.*, 2000). Selenium and zinc are present in high concentrations in the testes and epididymis of boars, and play an important role in the production and maturation of spermatozoa (Marin-Guzman *et al.*, 2000). Lipid peroxidation a process under which oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double Supplementation with zinc increases daily spermatozoa production and reduces the proportion of abnormal spermatozoa. Zinc also has antioxidative properties and may also act to reduce the reactive oxygen species and hence increase fertility (Bray *et al.*, 1997). The addition of antioxidants is an important consideration in Kolbroek semen for protection against lipid

peroxidation (Chatiza *et al.,* 2018). Various products of lipid peroxidation, including malondialdehyde (MDA), isoprostanes, and 4-hydroxynonenal are important biomarkers of oxidative stress in tissues (Yoshida *et al.,* 2012). Information regarding the effect of selenium and zinc supplementation on lipid peroxidation MDA levels of LW × LR and Kolbroek are scarce. The MDA production is determined using thiobartituric acid (TBA) (Wilson *et al.,* 2004) and is widely used as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids (Ayala *et al.,* 2014). (Tavilani *et al*., 2008). (Colagar *et al.,* 2013). Surai (2006) reported that antioxidant protection plays a key role in maintaining the integrity of the spermatozoa membranes and their fertilizing ability. Selenium acts as a component of glutathione peroxidases (GPx) in seminal plasma, there by assisting in the protection of spermatozoa against oxygen radicals-induced damages (Kantola *et al*., 1988). The information regarding the importance of selenium and zinc supplementation in Kolbroek boar semen is scarce. There is need to determine the effect of selenium and zinc supplementation in boar seminal plasma and spermatozoa quality. Therefore, the objective of the current study was to determine the effect of selenium and zinc supplementation on biochemical protein in seminal plasma constituents and lipid peroxidation and semen quality of epididymis spermatozoa derived from slaughtered LW × LR and Kolbroek boars. It was hypothesized that interaction of selenium and zinc supplementation may improve the epididymis spermatozoa quality, seminal plasma constituent and lipid peroxidation in semen.

6.2 Materials and methods

6.2.1 Experimental study site

The study was conducted at the Agricultural Research Council, Animal Production Institute (ARC-API), in the Germplasm, Conservation and Reproductive Biotechnologies Unit in Irene, South Africa. The ARC-API is located at 25º55′ South; 28º 12′ East and is located in the

Highveld region of Republic of South Africa and situated at an altitude of 1525 m above see level. Procedures involving animals were evaluated and approved by the Animal Ethics Committee of ARC-API before the experiment could be conducted (Reference: APIEC16/037), during the period from November 2016 to February 2017.

6.2.2 Experimental boars and treatments

A total of 24 Large White × Landrace, average body weight 81.6 ± 1.7 kg and 24 Kolbroek boars, average body weight of 74.2 ± 0.6 kg and 7 to 8 months old were used in the present study. At the end of the experiment, all boars were slaughtered, epidydimal semen collection and testicular measurements taken. The pigs were fed for six months. The experimental boars were grouped by genotype and allocated four diets for the whole experimental period. A total mixed ration was formulated to supply 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg DM and 11.6 g lysine /kg which meet the requirements of growing pigs (NRC, 1998). The diets were as follows: low selenium (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ). Water was provided *ad libitum* to the boars during the experimental period. The pens were cleaned daily and the pigs were dipped in Triatix® (Amitraz) every two weeks against ectoparasites and were dewormed once a month against endo-parasites using Valbazen® (Albendazole). Ingredient and chemical composition of the diets shown in Table 3.1. All the boars were offered 2 kg per day in the morning until slaughter.

6.2.3 Semen evaluation

6.2.3.1 Semen sample collection

A total of 24 Large White × Landrace and 24 Kolbroek boars six per genotypes allocated into four treatmet. Epidydimal semen was collected after slaughtered from the testicles. Epidydimal semen samples were collected from head of the epididymis immediately after slaughtering through an incision made by a razor blade on the right and left testicles. The semen samples were collected into a graduated 15 ml tube. The semen samples were placed in well-insulated flasks maintained at a warm temperature (39˚C) before being transported to the laboratory.

6.2.3.2 Semen volume and pH

Semen volume was measured by using a graduated 15 mL tube and it was recorded in millilitres (mL). Semen pH was determined using a pH meter [(HANNA Instruments®, South Africa (Pty) (Ltd)]. The pH electro-rode was washed with sterile water and wiped with sterile paper towel before being inserted into the tube containing the semen sample for 30 seconds.

6.2.3.3 Spermatozoa motility

Spermatozoa motility was determined using a Sperm Class Analyser® (Microptic S.L, Barcelona). Five hundred microliters of Ham's F-10 (Sigma-Aldrich, South Africa) and5 µL of semen were mixed in a 1 mL graduated tube and incubated for 5 minutes at 37 ^{o}C. After incubation, 10 µL of extended semen was placed on a pre-warmed microscopic slide (Omron) adjusted at 39°C, mounted with a cover slip and examined (x 10) under a phase contrast microscope (Nikon, Japan). Spermatozoa motility was categorised as follows: progression percentage, total motility (TM) = is a sum of progressive and non-progressive motility; progressivemotility (PM) = spermatozoa that are moving forward; non-progressive motility

(NPM) = spermatozoa that are not moving forward. Average values of velocity parameters; Curvilinear velocity (VCL) = average velocity which measures a spermatozoa movement along its actual path (μm/s); straight-line velocity (VSL) = average velocity which measures a spermatozoa movement along a straight line from beginning to the end (μm/s); average path velocity (VAP) = average velocity of the smoothed cell path (μm/s); linearity (LIN) = linearity movement is a ratio of VSL/VCL (%); straightness (STR) = straight line movement is a ratio of VSL/VAP (%) ; and wobble (WOB) = wavering movement which is a ratio VAP/VCL (%).

6.2.3.4 Spermatozoa concentrations

Spermatozoa concentration was determined with a 6310 spectrophotometer (Figure 3.9) (Jenway, United Kingdom). A square cuvette was filled with 3 mL of sodium citrate solution and placed in a spectrophotometer for at least 30 seconds. Raw semen (15 μL) was added in a square cuvette containing the sodium citrate solution, again placed in a spectrophotometer in order to read the absorbance. The absorbance read was used to determine the final spermatozoa concentration with the aid of a formula: 201 × 25.97 × absorbance – 0.3. The final spermatozoa concentration was recorded in millions per millilitre spermatozoa concentration.

6.2.3.5 Spermatozoa membrane integrity

Spermatozoa membrane integrity was determined using a hypo-osmotic test. A semen volume of 0.1 mL was mixed with 1 mL hypo-osmotic solution and incubated at 37 ^{o}C for one hour. Following incubation, 7 μL of semen was placed on a glass slide, then smeared and evaluated under a phase contrast microscope (x 40) (BH - 2) (Olympus. Japan), at least 200 spermatozoa per slide were counted. Spermatozoa with swollen and coiled tail were considered intact. Host – were evaluated as intact the membrane integrity and Host + does not intact membrane integrity.

6.2.3.6 *Spermatozoa morphology*

The morphology was determined microscopically after staining the semen samples with Eosin Nigrosin stain on a slide. Boar semen was added to 20µL Eosin Nigrosin staining solution in a 0.6 Ml micro-centrifuge graduated tube and mixed gently. A drop of 5µL boar semen and Eosin Nigrosin stain was placed on a clear end of a microscope slide and smeared. This staining method indicates the percentage live or dead spermatozoa and allows an effective evaluation of the spermatozoa morphology (normal or abnormal). The spermatozoa smears were prepared on a clean, warm microscope slide to avoid temperature shock to the spermatozoa and evaluated on the same day of semen collection with the aid of a fluorescent microscope (BX 51TF) using an oil immersion objective (× 100 magnification). Live spermatozoa were further evaluated for spermatozoa morphology and abnormalities. Abnormalities of the spermatozoa were categorised as primary (small, large or swollen head, double heads, abnormal acrosome, elongated and mid-piece, double and short tail), secondary (detached, loose or damaged acrosomes, bent and protoplasmic droplets of the mid-piece, bent and shoe-hook tail) and tertiary abnormalities (reacted acrosomes and coiled tails).

6.2.4 Lipid peroxidation using the MDA levels

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production, using thiobartituric acid (TBA) (Suleiman *et al*., 1996). The MDA level was measured in the spermatozoa and semen plasma. The spermatozoa pellets that resulted from separating the seminal plasma from semen by centrifugation at 15000 ×g for 10 minutes was re-suspended in 2 mL phosphate-buffered saline PBS (pH 7.2) or variable volume to obtain a spermatozoa concentration of 10 $\times 10^6$/mL. Lipid peroxidation was measured in spermatozoa after the addition of 2 mL of TBA reagent (15 % w/v trichloroacetic acid and 0.25 NHCL) to 1 mL of

spermatozoa suspension. The mixture was treated in a boiling water bath for 15 minutes after cooling, the suspension was centrifuged at 1500 × g for 10 minutes, the supernatant was then separated and absorbance was measured at 535 nm by spectrophotometer. The MDA concentration was determined by the specific absorbance coefficient (1.56×10^5moL/cm^3). The same procedure was performed to measure MDA in semen plasma (Suleiman *et al.*, 1996).

6.2.5 Determination of biochemical protein for seminal plasma

Seminal plasma was collected from all the semen samples by means of centrifugation at 1500 rpm for 10 minutes for biochemical analysis. Following centrifugation, seminal plasma was removed using 1 mL disposable plastic pipettes. The seminal plasma was then transferred into 5 mL centrifuge tubes and stored at -20 ºC until they were analysed at the University of Pretoria Pathology Laboratory. Due to higher costs of determining seminal plasma constituents, twenty-four samples per genotype were randomly selected for evaluation. For the determination of sodium, potassium, magnesium, zinc, phosphorus, protein and calcium, seminal plasma was centrifuged at 1500 rpm (4 °C) for 20 minutes. Then 1 M sodium hydroxide was added to form a coloured salt complex. The concentration elements in the coloured complex solution were determined spectrophotometrically (fluorescent colour intensity) using a digital fluorescent microscope.

6.2.6 Statistical analyses

The interaction of selenium, zinc and genotype on epididymis spermatozoa quality, seminal plasma constituents and lipid peroxidation were performed using (SAS) version 9.3 statistical software (SAS, 1999). The GLM procedure was also used to determine the effect on interaction

of selenium, zinc and genotype. A 5% significance level was used. The model for spermatozoa morphology, motility and velocity, seminal plasma constituents and lipid peroxidation was:

$Y_{ijkl} = \mu + S_i + Z_j + G_k + (S \times Z)_{ij} + (S \times G)_{ik} + (Z \times G)_{jk} + (S \times Z \times G)_{ijk} + E_{ijkl}$

Y_{ijkl} = epididymis spermatozoa quality, seminal plasma constituent and lipid peroxidation.

μ = is the overall mean common to all observations

S_i = Selenium level (i = LS, HS)

Z_j = Zinc level (j = LZ, HZ).

G_k =Genotype (k = Kolbroek, Large White × Landrace)

$(S \times Z)_{ij}$=selenium × zinc is interaction

$(S \times G)_{ik}$ =the interaction of Selenium ×genotype

$(Z \times G)_{jk}$= the interaction of Zinc × genotypes

$(S \times Z \times G)_{ijk}$= the interaction of selenium × zinc × genotype

E_{ijkl}= the residual error.

6.3 Results

6.3.1 Effect of genotype, selenium and zinc supplementation on semen volume and spermatozoa concentrations

The effect of selenium and zinc supplementation on semen volume, semen pH and spermatozoa concentration in Large White × Landrace and Kolbroek boars are shown in Table 6.1. There were no effects of genotype, selenium and zinc supplementation on semen volume and pH ($P > 0.05$). No interactions existed on semen volume ($P>0.05$). There was an interaction of genotype and selenium inclusion on semen pH ($P<0.05$). There was no genotype, selenium and zinc effects on spermatozoa concentration ($P>0.05$).

Table 6. 1: Least square means for Large White × Landrace boars and Kolbroek boar semen volume, semen pH and spermatozoa concentration fed on different levels of selenium and zinc

Genotypes	Diets		Semen parameters		
	Selenium (mg/kg)	Zinc (mg/kg	Semen volume (mL)	Semen pH	Spermatozoa concentration ($\times 10^6$/mL)
LW×LW	High	High	8.10	6.83[a]	0.458
	High	Low	8.70	6.78[a]	1.839
	Low	High	8.38	7.03[b]	1.351
	Low	Low	8.13	7.00[b]	1.298
	SEM		0.402	0.081	1.068
Kolbroek	High	High	8.41	7.00[b]	0.825
	High	Low	8.08	7.00[b]	1.042
	Low	High	7.58	6.87[a]	2.369
	Low	Low	8.33	7.00[b]	1.946
	SEM		0.491	0.121	0.687
	Genotype		NS	NS	NS
	Selenium		NS	NS	NS
	Zinc		NS	NS	NS
	Se × zn		NS	NS	NS
	Genotype × Se		NS	*	NS
	Genotype × Zn		NS	NS	NS
	Genotype × Se × Zn		NS	NS	NS

[abc]Values with different superscripts within a column differ significantly (P<0.05) ***P<0.001, **P<0.01, *P<0.05 and NS: not significant

Diets = low selenium (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65 mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ).

6.3.2 Effect of genotype, selenium and zinc concentrations on spermatozoa morphology

The effect of selenium and zinc supplementation on spermatozoa morphology for Large White × Landrace and Kolbroek boars is shown inTable 6.2. There was no effects of genotype onlive spermatozoa percentages ($P > 0.05$). There was an interaction between genotypes and selenium concentration on live spermatozoa concentration ($P<0.05$). Live spermatozoa concentration was higher in crossbred boars fed on high selenium concentration ($P<0.05$). There was a significant selenium and zinc supplementation on the percentage of dead spermatozoa ($P<0.05$). Crossbred pigs fed low selenium and low zinc concentration had the highest percentage of dead spermatozoa ($P < 0.05$).

There were genotype and zinc effects on proximal droplets percentage ($P<0.05$). There was, however a genotype × selenium interaction on proximal droplets percentage ($P < 0.05$). There was no genotype, selenium and zinc effects on distal droplet percentage ($P>0.05$). There was an effects of genotype, selenium and zinc on head abnormalities of spermatozoa ($P<0.05$). Selenium and zinc supplementation influenced on head abnormalities of spermatozoa (Table 6.2).

6.3.3 Efect of genotype, selenium and zinc concentrations on spermatozoa motility

Selenium and zinc supplementation had no effects on spermatozoa motility (Table 6.3). The spermatozoa motility was not affected by genotypes ($P>0.05$).

Table 6.2: Effect of selenium and zinc supplementation on spermatozoa morphology for Large White × Landrace boars and Kolbroek boars

Genotype	Selenium	Zinc	Spermatozoa morphology %						
			Live	Dead	Proximal droplets (%)	Distal droplet	Head	Midpiece	Tail
LW × LR	High	High	77.5^{a}	18.5^{bc}	14.8^{ab}	7.8^{ab}	0.3^{d}	0.3^{c}	4.3^{a}
	High	Low	77.8^{a}	20.3^{bc}	3.7^{c}	4.3^{b}	2.2^{cd}	0.5^{c}	7.7^{a}
	Low	High	61.2^{c}	25.5^{b}	13.7^{ab}	10.0^{ab}	5.8^{b}	12.2^{a}	8.0^{a}
	Low	Low	63.1^{bc}	37.7^{a}	10.7^{b}	13.3^{a}	10.0^{a}	6.8^{ab}	8.3^{a}
	SEM		21.38	10.59	8.547	4.179	16.23	5.63	6.860
Kolbroek	High	High	59.0^{c}	28.2^{ab}	17.3^{a}	13.5^{a}	10.5^{a}	5.0^{bc}	10.7^{a}
	High	Low	71.3^{ab}	14.5^{c}	10.7^{b}	14.0^{a}	8.5^{ab}	3.5^{bc}	6.3^{a}
	Low	High	71.5^{ab}	18.5^{bc}	9.0^{bc}	12.0^{a}	5.2^{bc}	3.2^{bc}	5.7^{a}
	Low	Low	136.17^{abc}	22.3^{bc}	10.0^{bc}	11.8^{a}	7.0^{ab}	4.8^{bc}	11.0^{a}
	SEM		12.1223	5.1419	5.88	3.72	2.81	1.974	5.53
	Genotype		NS	**	*	NS	**	**	NS
	Selenium		*	NS	NS	NS	*	*	NS
	Zinc		NS	NS	**	NS	*	NS	*
	Genotype × selenium		***	**	*	*	***	***	NS
	Genotype × zinc		NS	*	NS	NS	NS	NS	NS
	Genotype × Se × zn		NS	NS	NS	NS	NS	**	NS

[a,b]Values with different superscripts within a column differ significantly (P<0.05) ***P < 0. 001, **P < 0.01, *P<0.05 and NS not significant

[2]diets= low selenium (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ).

6.3.4 Effects of genotype, selenium and zinc supplementation on spermatozoa velocity

The effects of selenium and zinc supplementation on spermatozoa velocity of Large White × Landrace and Kolbroek boar semen are shown in Table 6.4. Neither the supplementation of selenium and zinc, nor the genotype effect on VCL ($P<0.05$). There was, however, a selenium and zinc interaction on VSL ($P<0.05$). In both breeds, VAP was lowest in boars fed on diets with low levels of zinc ($P<0.05$). The VAP was affected by levels of selenium and zinc supplementation of both genotypes ($P<0.05$). Kolbroek pigs fed on low selenium level had higher LIN than those on low selenium diets irrespective of zinc levels. Selenium and zinc supplementation had had no effect on WOB and ALH in both genotypes ($P>0.05$). All three variables influence the BCF ($P<0.05$). All two way interactrions were also significant on BCF ($P<0.05$).

6.3.5 Effect of genotype, selenium and zinc supplementation on mineral concentrations in semen

Mineral concentrations in seminal plasma in Large White × Landrace and indigenous Kolbroek boars are showed in Table 6.5. Potassium and calcium concentrations in the seminal plasma constituents were no effects by inclusion levels of selenium and zinc supplementation in both Large White × Landrace and Kolbroek boar ($P >0.05$). There was an effects of selenium and zinc supplementation on selenium and sodium in seminal plasma constituents ($P <0.05$). Magnesium concentrations in the seminal plasma constituents was increased when the inclusion levels of selenium and zinc supplementation increased in both genotypes ($P <0.05$). Phosphorus concentrations in the seminal plasma constituents was increased, the inclusion levels of selenium and zinc supplementation increased of Large White × Landrace and Kolbroek boar ($P< 0.05$).

Table 6.3: Effect of dietary selenium and zinc supplementation on epididymis spermatozoa motility of Large White × Landrace and Kolbroek boar semen

Genotypes	Selenium	Zinc	TM (%)	PM (%)	NPM (%)	STC (%)	Rapid (%)	Medium (%)	Slow (%)
LW×LR	High	High	91.71	61.7	37.9	9.60	62.7	24.2	3.6
	High	Low	88.27	54.0	39.6	18.7	61.0	17.2	3.0
	Low	High	89.87	54.86	45.0	11.3	59.9	24.7	3.7
	Low	Low	92.93	39.45	57.7	12.4	61.3	23.3	2.6
	SEM		2.87		6.52	6.19	23.58	5.85	2.05
KB	High	High	88.90	39.73	49.2	11.1	63.6	21.5	3.8
	High	Low	90.38	42.50	47.9	9.6	67.7	20.2	2.5
	Low	High	93.52	42.23	51.3	6.5	60.4	29.3	3.8
	Low	Low	92.40	43.87	48.5	22.8	52.7	32.7	4.4
	SEM		8.98	8.35	7.98	6.19	23.91	8.36	3.63
	Breed		NS	NS	NS	NS	NS	NS	NS
	Selenium		NS	NS	NS	NS	NS	NS	NS
	Zinc		NS	NS	NS	NS	NS	NS	NS
	Genotype× Se		NS	NS	NS	NS	NS	NS	NS
	Genotype×Se		NS	NS	NS	NS	NS	NS	NS
	Selenium × zinc		NS	NS	NS	NS	NS	NS	NS
	Genotype×Se×Zn		NS	NS	NS	NS	NS	NS	NS

[abc]Values with different superscripts within acolumn differ significantly (P<0.05); ***P<0.001, **P <0.01, *P<0.05 and NS not significant
, NPM- non-progressive motility, PM -progressive motility, TM- total motility, Rapid -percentage of rapidly moving spermatozoa, Static- percentage of static spermatozoa; Diets= low selenium (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ).

Table 6.4: Effect of dietary selenium and zinc supplementation on velocity parameters of Large White × Landrace and Kolbroek boar semen

Genotypes	Selenium	Zinc	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	LIN (%)	STR (%)	WOB (%)	ALH (µm)	BCF (Hz)
LW×LR	High	High	145.4	28.5^{b}	60.4^{b}	21.7^{ab}	49.3^{ab}	45.3	4.4	7.9^{bc}
	High	Low	160.0	37.6^{a}	84.7^{a}	22.9^{ab}	50.6^{ab}	46.4	4.8	8.9^{bc}
	Low	High	131.5	28.2^{b}	56.4^{b}	21.9^{ab}	50.7^{ab}	43.3	4.1	6.9^{c}
	Low	Low	166.8	32.0^{ab}	67.3^{b}	19.4^{b}	47.7^{ab}	40.6	4.7	10.1^{ab}
	SEM		10.57	7.16	15.46	2.62	3.81	2.00	0.90	3.87
Kolbroek	High	High	163.3	32.3^{ab}	67.9^{b}	19.9^{ab}	47.2^{b}	41.8	4.3	12.9^{a}
	High	Low	168.2	36.2^{a}	70.6^{ab}	22.0^{ab}	51.7^{ab}	42.3	4.4	12.9^{a}
	Low	High	158.1	35.6^{ab}	67.3^{b}	23.3^{a}	53.7^{a}	40.4	4.2	10.6^{ab}
	Low	Low	135.2	31.3^{ab}	61.0^{b}	23.2^{a}	49.7^{ab}	42.6	3.7	9.7^{bc}
	SEM		37.53	7.06	14.15	1.30	2.24	3.22	1.15	2.00
	Genotype		NS	NS	NS	NS	NS	NS	NS	*
	Selenium		NS	NS	NS	NS	NS	NS	NS	**
	Zinc		NS	NS	**	NS	NS	NS	NS	**
	G x Se		NS	NS	NS	*	NS	NS	NS	**
	G x Zn		NS	NS	*	NS	NS	NS	NS	*
	Se × Zn		NS	**	**	NS	*	NS	NS	*
	G×Se×Zn		NS	**	**	NS	NS	NS	NS	NS

[abc] Values with different superscripts within a column differ significantly (P<0.05); *** P < 0.001, **P < 0.01, *P<0.05 and NS not significant

ALH= Amplitude of lateral head, BCF= Frequency with which the spermatozoa track crossed the spermatozoa path, LIN-linearing = Average value of the ratio, VAP –velocity average pathway, VSL -velocity curvilinear; Diets = low selenium; Se = selenium; Zn = zinc; G= genotype; (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ).

Table 6.5: Effect of dietary selenium and zinc supplementation on biochemical variables in seminal plasma in Large White × Landrace (n = 24) and indigenous Kolbroek (n = 24) boars

Biochemical analysis[1]									
Genotype[2]	Selenium mg/k	Zinc mg/kg	Zn mg/L	Se mg/L	Na mg/L	K mg/L	Mg mg/L	P mg/L	Ca mg/L
LW × LR	High	High	22.1^{a}	166.6^{ab}	184.4^{ab}	227.3	182.3^{b}	467.7^{ab}	201.1
	High	Low	28.5^{a}	144.4^{b}	244.8^{ab}	215.4	220.3^{ab}	471.5^{ab}	167.2
	Low	High	30.3^{a}	157.3^{b}	254.3^{a}	238.6	166.1^{ab}	493.1^{a}	155.9
	Low	Low	25.7^{a}	158.1^{b}	155.7^{b}	279.9	228.7^{b}	422.4^{b}	206.1
	SEM		4.89	9.91	49.00	32.71	64.79	47.45	19.31
Kolbroek	High	High	30.0^{a}	209.0^{a}	212.3^{ab}	244.0	265.7^{a}	481.8^{a}	203.2
	High	Low	20.6^{a}	225.5^{a}	195.6^{ab}	222.3	215.0^{ab}	469.3^{ab}	197.4
	Low	High	25.5^{a}	163.5^{ab}	246.3^{a}	215.8	225.6^{ab}	497.5^{a}	187.8
	Low	Low	31.2^{a}	177.9^{ab}	229.0^{ab}	230.2	517.0^{b}	479.4^{a}	191.5
	SEM		4.86	0.86	72.29	60.27	78.27	50.75	57.19
	Genotype		NS	*	NS	NS	NS	NS	NS
	Selenium		NS	NS	NS	NS	NS	NS	NS
	Zinc		**	NS	NS	NS	*	NS	NS
	Se × Zn		NS	NS	NS	NS	**	NS	NS
	G × Se		**	NS	**	NS	NS	NS	NS
	G × Zn		*	NS	NS	NS	*	NS	NS
	G ×Se × Zn		*	NS	NS	NS	**	*	NS

^{abc}Values with different superscripts within a column differ significantly (P<0.05), ***P < 0.001, **P < 0.01, * P<0.05 and NS not significant, [1]Mg=magnesium, P=phosphorus and Ca= calcium, Zn=zinc, Se=selenium, Na=sodium, K=potassium, Mg=magnesium, P=phosphorus and Ca= calcium, G = genotype

[2] Genotypes LW × LR = Large White × Landrace and Kolbroek

Diets = low selenium (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ).

6.3.5 Effects of genotype, selenium and zinc concentrations on hyper osmotic swelling test

The effect of inclusion levels of selenium and zinc supplementation on hyper osmotic swelling test in Large White × Landrace and Kolbroek boars are presented in Table 6.6. There was effect of selenium and zinc supplementation on HOS+ of both genotypes ($P < 0.05$). Large White × Landrace boars that were fed high levels of both selenium and zinc supplementation had higher HOS+ ($P < 0.05$) than those fed low levels. Kolbroek boars that were fed high inclusion levels of selenium had higher HOS+ ($P < 0.05$). Large White × Landrace boars that were fed high inclusion levels of selenium had lower HOS- ($P < 0.05$), Kolbroek boars fed high inclusion levels of selenium, had higher HOS- ($P<0.05$) than those on low levels.

6.3.6 Effects of genotype, selenium and zinc concentrations on lipid peroxidation

The effect of inclusion levels of selenium and zinc supplementation on lipid peroxidation levels in Large White × Landrace and Kolbroek boars are illustrated in Figure 6.1. Large White × Landrace boars that fed on low levels of both selenium and zinc had lower levels of MDA ($P<0.05$) than boars that fed on diets containing high inclusion levels of selenium and zinc. In Kolbroek boars that were fed on low levels of both selenium and zinc, the levels of MDA were higher ($P<0.05$) than those fed on low selenium and zinc levels. Selenium and zinc supplementation affected MDA levels in both genotypes ($P<0.05$).

Table 6.6: Effects of dietary selenium and zinc supplementation on hyper osmotic swelling test (HOST) in Large White × Landrace (n = 24) and indigenous Kolbroek (n = 24) boars

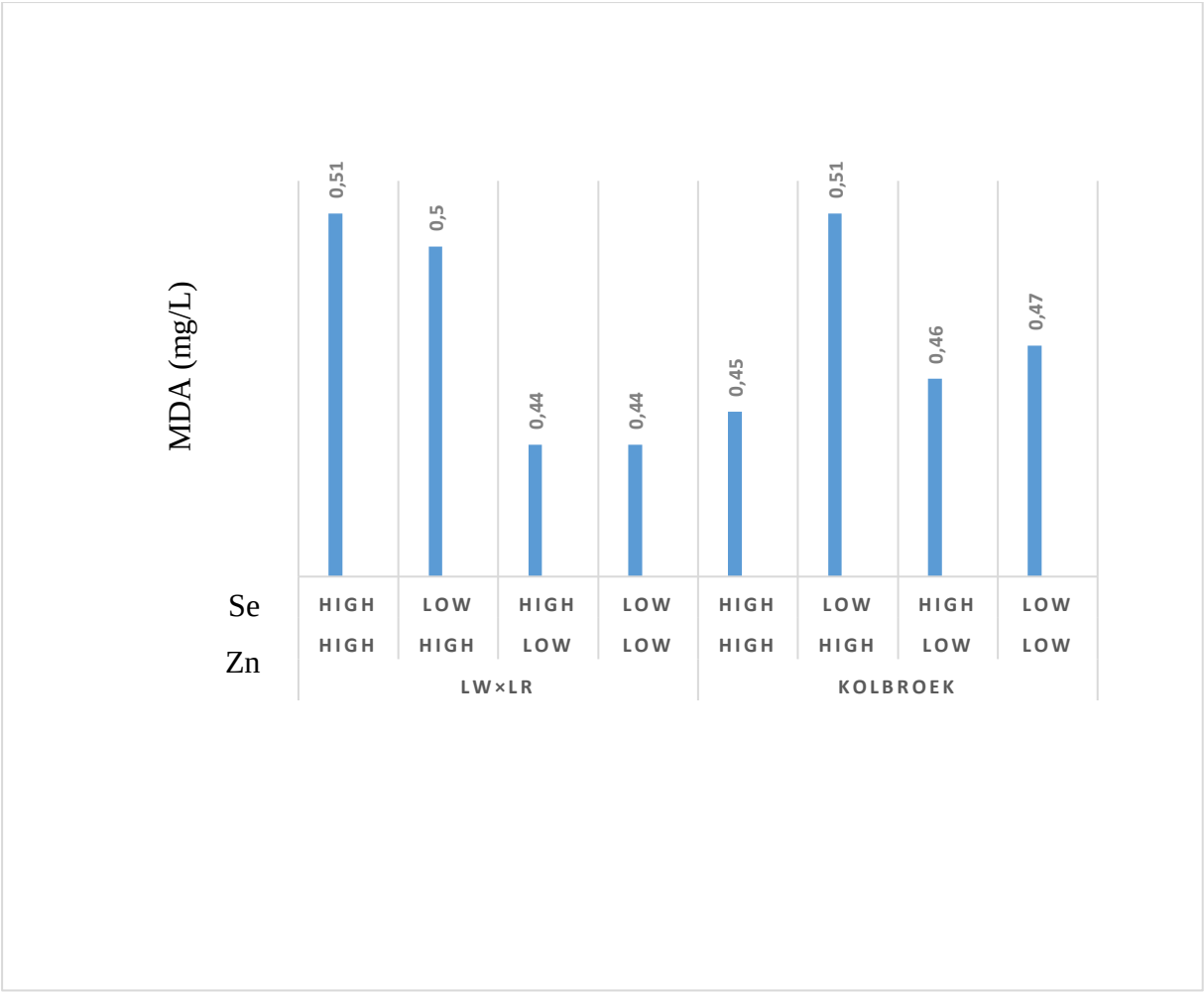
	Diets[2]		Variables[3]	
Genotypes[1]	Selenium (mg/kg)	Zinc (mg/kg	HOS+	HOS-
LW×LR	High	High	157.3^{ab}	42.7^{ab}
	High	Low	166.2^{a}	33.8^{b}
	Low	High	145.0^{b}	55.0^{a}
	Low	Low	152.5^{ab}	46.2^{ab}
Kolbroek	High	High	144.5^{b}	55.5^{a}
	High	Low	151.8 ^{ab}	48.2^{ab}
	Low	High	144.8^{b}	53.5^{a}
	Low	Low	140.5^{b}	59.5^{a}
	SEM		6.8465	6.4299
	Genotype		NS	NS
	Selenium		*	*
	Zinc		NS	NS
	Genotype×selenium		NS	NS
	Genotype×zinc		NS	NS
	Selenium×zinc		NS	NS
	Genotype×selenium ×zinc		NS	NS

[abc] Values with different superscripts within a columm differ significantly (P<0.05), ***P<0.001, **P<0.01, *P<0.05 and NS: not significant

[1]genotypes = (LW×LR) Large White × Landrace; Kolbroek

[2]diets= low selenium (0.26 mg/kg), low zinc (0.35 mg/kg) (LSLZ); low selenium (0.26 mg/kg), high zinc (0.74 mg/kg) (LSHZ); high selenium (0.65mg/kg), low zinc (0.35 mg/kg) (HSLZ) and high selenium (0.65 mg/kg), high zinc (0.74 mg/kg) (HSHZ).

[3]variables HOST+ and HOST-



Fugure 6.1: The effect of inclusion levels of selenium and zinc supplementation on lipid peroxidation in Large White × Landrace (n = 24) and indigenous Kolbroek (n = 24) boars

MDA-Malondialdehyde, mg/L-milligram/litter, Se = selenium and Zn = zinc
Low selenium low zinc (HSLZ) (0.65, 0.35 mg/kg), high selenium high zinc (HSHZ) (0.65, 0.74 mg/kg), low selenium high zinc (LSHZ) (0.26, 0.74 mg/kg) and low selenium low zinc (LSLZ) (0.26, 0.35 mg/kg)

6.4 Discussion

Zinc has an important role in many physiological functions, such as reproduction and growth of animals (Atakisi *et al*., 2009). The present study showed that selenium × zinc supplementation of both genotypes affected epididymis spermatozoa quality, biochemical protein in seminal plasma constituent and lipid peroxidation althogh these traits were within the normal range (5 and 10 mg/L). Generally, spermatozoa quality increased mainly due to the reduced number of spermatozoa with abnormal head shapes, decreased motility, and abnormal acrosomes (Parrish *et al.,* 2017). Seminal plasma represents the seminal fraction with the greatest antioxidant capacity; its removal renders spermatozoa vulnerable to lipid peroxidation due to depletion of factors responsible for the uptake of reactive oxygen species (Torres *et al.,* 2016).

The findings that the semen pH was not influenced by supplementation of selenium and zinc in LW × LR and Kolbroek pigs was unexpected. The semen pH was not influenced by pig genotype. The semen pH increased as levels of selenium supplementation increased in boars (Speight *et al.,* 2010). Jacyno *et al*. (2002) reported that semen pH of boar spermatozoa fraction was 7.69. Boar semen pH increased with selenium supplementation in pigs (Vyt *et al.*, 2004). Surai and Fisinin (2015) reported that increases in semen pH in pigs supplemented with selenium. Studies on zinc supplementation on semen pH have been reported in crossbred cattle (Kumar *et al.*, 2006). No information on interaction of selenium × zinc influence the semen pH of Large White × Landrace and Kolbroek pigs. Therefore, future research should further explore these aspects.

Semen volume was not influenced by supplementation of selenium and zinc in LW × LR and Kolbroek pigs. Jacyno *et al*. (2002) reported that boars fed on diets supplemented with

selenium had high semen volumes. Kolodziej and Jacyno (2005) reported increases in semen volume with selenium supplementation in boars. Cheah and Yang, (2011) reported increases in semen volume in pigs supplemented with zinc. Boars supplemented with selenium exhibited a higher volume of ejaculate (Martins *et al*., 2018). Boars receiving increased levels of selenium in the diet exhibited a marked increase in semen volume ejaculate (Marin-Guzman *et al.* 1997). Speight *et al.* (2012) reported that semen volume ejaculate decreased with the increase in selenium in the diet. Boars fed on zinc supplements had increased semen volume (Cheah and Yang, 2011). Ogbu *et al.* (2016) reported increases in semen volume in turkeys supplemented with both selenium and zinc. Tthere is no data on how does selenium × zinc influence the semen volume of Large White × Landrace and Kolbroek pigs.

Spermatozoa concentration was not influenced by supplementation of selenium and zinc in LW × LR and Kolbroek pigs. Surai and Fisinin (2015) reported that increasing selenium supplementation increased spermatozoa concentration of boars. Spermatozoa concentration increased as levels of selenium and zinc supplementation in pigs (Villaverde *et al.,* 2014; Horký *et al.,* 2011). Lopez *et al.* (2010) reported that boars fed on a diet supplementated with selenium had increases in spermatozoa concentration. At low level of selenium, the spermatozoa concentration increased and high level started decreasing, suggesting that it is likely to play an important role for the production and maturation of spermatozoa (Marin-Guzman *et al.,* 1997; 2000). Kaur and Parshad (2005) reported an increase in spermatozoa concentration in pigs supplemented with selenium. Marin-Guzman *et al.* (2000b) reported an increase in spermatozoa concentration in pigs supplemeted with selenium. Horký *et al.* (2012) reported that dietary supplementation with selenium increased spermatozoa concentration. Speight *et al.* (2012) reported that increasing selenium decreased spermatozoa concentration in pigs. Spermatozoa concentration also increased as inclusion levels of selenium in the diet increased

in pigs (Martins *et al*., 2018). Ogbu *et al*., (2016) reported further increases in spermatozoa concentration when turkey were supplemented with both with selenium and zinc. There is no information on how does the interaction of selenium × zinc influence the spermatozoa concentration of Large White × Landrace and Kolbroek pigs.

The number of live and dead spermatozoa were affected by selenium and zinc suppplementation in Large White × Landrace and Kolbroek pigs. At low level of selenium, the number of live spermatozoa was high but decreased at high concentration, suggesting the high levels selenium could be toxic. Kaur and Parshad (1994) reported that boars fed on diets containing selenium increased spermatozoa concentration, motility, and percentage of live spermatozoa. At low level of selenium, the proximal droplets increased and at high level started decreasing, suggesting the high levels selenium are toxic. The increase in proximal droplets results in semen with abnormal spermatozoa morphology. Merrells *et al.* (2009) reported that increases zinc supplementation caused decreased spermatozoa abnormalities of head and tail. Martins *et al.* (2018) reported increases in abnormal head and proximal droplets in pigs supplemented with selenium. Speight *et al.* (2015) reported that the increases in percentage of spermatozoa abnormal heads had increased with selenium supplementation in boars. Lovercamp *et al.* (2013) reported that boars fed on diets with increasing selenium and zinc increased spermatozoa, head and tail abnormalities. The tail spermatozoa abnomalities decreased at low levels. Zinc increases intergrity of plasma membrane of boars or reduce stress related to decline in androgen production by testicles (Marin-Guzman *et al*., 2000a; Thundathil *et al*., 2001). Therefore, Suraiand Fisinin (2015) reported that the low selenium diet caused changes in spermatozoa of boars. In fact, abnormal spermatozoa head percentage and proximal droplet percentage significantly decreased, but abnormal tail percentage increased due to selenium dietary supplementation (Surai and Fisinin, 2015). Furthermore, spermatozoa

concentration decreased and percentage of immature spermatozoa with cytoplasmic droplets increased when boars were fed on a low-Se diet (Marin-Guzman *et al*., 2000). Added selenium in the diet enhanced spermatozoa motility and decreased the proportion of cells with cytoplasmic droplets and bent and shoehook tails (Marin-Guzman *et al.*, 1997). Boars that received selenium supplementation had an increased percentage for abnormal tails (Martins *et al.*, 2018). Surai and Fisinin (2015) reported that selenium supplementation in the diet of boars increases spermermatozoa concentration but reduced straight forward movement of the spermatozoa. There is no information is available in LW × LR and Kolbroek pigs.

The findings showed coiled tail spermatozoa were not influenced by selenium and zinc inclusion, which could be explained by the ability of selenium and zinc to scavenge the free radicals from damaging spermatozoa. Surai and Fisinin (2014) reported that supplementation with selenium had lower bent and shoehook tails in boar. Broekhuijse *et al*. (2012) reported that decreased motility during storage may result from functional abnormalities of spermatozoa mitochondria. In addition, there is no information on how does interaction of selenium and zinc influence the coiled tail spermatozoa of Large White × Landrace and Kolbroek pigs.

Spermatozoa motility was not influenced by inclusion levels of selenium and zinc supplemetation in the diet. There was no effect of genotype on spermatozoa motility. Tareq *et al.* (2012) reported that motility and viability increased significantly with selenium supplementation. Kaur and Parshad (1994) reported boar fed dietary levels of selenium increased motility spermatozoa. Surai and Fisinin, (2015) reported that the spermatozoa-rich fractions of ejaculates with >85 % motillty spermatozoa. Martins *et al.* (2015) reported that increasing inclusion levels of selenium had an increased on total and progressive motility in pigs. The finding that PM, VSL, VAP, LIN, STR, WOB and BFC were not influenced by

inclusion levels of selenium and zinc supplementation. Selenium supplementation has been shown to increase progressive %, VAP, VSL, VCL, STR, LIN, ALH, BCF Pietrain boars (Lopez *et al*., 2010). Zinc supplementation caused decreases in semen quantity or quality in pigs (Althouse *et al.*, 2000). Cheah and Yang (2011) reported increases in spermatozoa motility in pigs supplemented with increasing levels of zinc. There is evidence of increases in PM, VSL, VCL, VAP, LIN, STR, WOB and BFC on supplementation with selenium and zinc in indigenous turkeys (Ogbu *et al*., 2016). No data is available in Kolbroek pigs. It would, therefore, be of interest for further understanding the role of selenium × zinc interaction on spermatozoa motility quality in pigs.

The findings showed no influence of selenium and zinc inclusion to zinc constituents of both Large White ×Landrace and Kolbroek boars suggesting that zinc constituents are within normal range and can be used as an accurate index for the evaluation of the efficiency of seminal protein constituents. At low level of selenium, the zinc constituents were high, suggesting the high levels zinc are toxic. At high level of selenium, the zinc constituents increased suggesting the low levels zinc are toxic. The findings that the Kolbroek boars fed on diets with low selenium, sodium concentration was increased, however, at high inclusion levels of zin, c seminal plasma constituents also increased. The sodium constituent was affected by selenium and supplementation. There was an effect of supplementation on seminal plasma of Large White × Landrace pigs. At low level of selenium was high and at low levels sodium concentration was low suggesting the high levels zinc are toxic. The increased sodium constituents in seminal plasma could be due to a decreases the number of spermatozoa (Rodríguez *et al.*, 2012). At low levels of both selenium and zinc, K constituents for seminal plasma was high suggesting that the high levels zinc are toxic. The inclusion levels of selenium and did not affect K of both LW × LR and Kolbroek boars. Ogbu *et al*. (2016) reported that

incrasing of inclusion levels of selenium and zinc, Na, K in seminal plasma constituent's values increased in indigenous turkey semen. No data is available in Kolbroek pigs.

Magnesium seminal plasma constituents was affected by selenium and zinc supplementation in LW × LR boars. At low levels of selenium, magnesium and calcium constituents was increased and at high level of selenium started decreasing, this suggest the low levels zinc are toxic. It could be increase magnesium may be due to the ability of magnesium constituents to reduce the activity of lipogenic enzymes or raise the activity of lipoprotein lipase (Rayssiguier *et al.,* 1991). The inclusion levels of selenium and zinc affect potasium and calcium of Large White × Landrace and Kolbroek boars. At low level of selenium, potassium constituents for seminal plasma increase and at high level of zinc started decreasing, this suggest the high levels zinc are toxic. It could be increase phosphorous constituents due to maturation and fertilizing capacity until passage through the epididymis. Olatunbosun *et al*. (2013) reported that the calcium constituents stimulate immature spermatozoa whereas in ejaculated semen, it inhibits spermatozoa motility. Holger *et al*., (2004) reported that increasing of inclusing levels of selenium and zinc, semen quality and biochemical constituents increased in turkey. Pipan *et al.* (2017) reported that increasing inclusion levels of selenium, seminal plasma constituent levels and spermatozoa quality increased. Surai and Fisinin (2015) reported that increasing of increasing selenium, seminal plasma levels increased. Little information is available in Kolbroek pigs.

The MDA level was affected by selenium and zinc supplementation of LW x LR and Kolbroek boar. There was no effect of genotypes on lipid peroxidation MDA levels of Large White × Landrace pigs. Lopez *et al* (2010) reported that the decreases in concentration of MDA levels

in Pietrain boars supplemented with increasing levels of selenium. The findings suggest that the selenium may be more available to be utilized by the GPX enzyme for protection of the PUFAs in the spermatozoa plasma membrane from lipid peroxidation (Cerolini *et al.*, 2000). Lovercamp *et al.* (2013); Liu *et al.* 2016) reported that increasing of inclusion levels of selenium, lipid peroxidation increased in boar. Surai and Fisinin (2014) reported increases in lipid peroxidation MDA levels in pigs supplemented selenium. Boars fed diet with addition of zinc supplementation had increases lipid peroxidation (Alsalman *et al*., 2013); in quail fed with supplementation zinc had increased the lipid peroxidation on the semen quality (Sahin *et al.*, 2009). However, studies on increases lipid peroxidation MDA on supplementation with selenium × zinc in goat (Kumar *et al*., 2013). There is little information available in Large White × Landrace and Kolbroek pigs.

The hypo-osmotic swelling test (HOST) + and HOST – were not affecte by selenium and zinc supplementation of LW × LR boars. Roy *et al.* (2013) reported that increasing inclusion levels of zinc, HOST spermatozoa increased in pigs. There was no effect of genotypes on HOST + and HOST of Large White × Landrace pigs. At high level of selenium, the HOST + increased and at low level of selenium started to decreasing, this suggest the low levels zinc are deficiency. It could be HOST + increase due to increased membrane acrosome integrity. Foxcroft *et al.* (2008) reported that the Hypo-osmotic swelling test resistance of the boar spermatozoa was correlated with fertility. Kumar *et al.*, (2014) thus, increase in antioxidative status might be responsible for increasing acrosome integrity and the reactive oxygen species which are continuously produced in spermatozoa membrane. However, studies on zinc supplementation on HOST spermatozoa have focused on crossbred cattle (Kumar *et al.*, 2006). Little is known on how does interaction of selenium × zinc influence the semen HOST + and HOST of Large White × Landrace and Kolbroek pigs.

6.5 Conclusions

In conclusion, the combination of selenium and zinc were increased in the spermatozoa traits, expect PM, NPM, VSL, VAP, LIN, STR, WOB, BCF and HOST of both genotypes. Seminal plasma of zinc, K and Ca were not affected by supplementation of selenium and zinc, expect selenium, Na, Mg and P constituents of both genotypes. Supplementation of seleniumand zinc reduced semen lipid peroxidation MDA levels of both genotypes. Therefore, dietary levels of selenium and zinc supplementation prevents spermatozoa with MDA levels, directly leading to the improvement of motility parameters in Large White × Landrace and Kolbroek boars. Therefore, it concluded that selenium and zinc combination did not increased the semen quality which may result in overall improvement in reproductive performance of Large White × Landrace and Kolbroek pigs.

6.6 References

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Chapter 7

Interaction of selenium × zinc supplementation on testicular and accessory sex gland morphology and spermatogenesis in Large White × Landrace and Kolbroek boars

Abstract

The study was designed to determine the interaction of selenium and zinc on testicular and accessory sex gland morphology and spermatogenesis of Large White × Landrace (LW × LR) (n=24) and Kolbroek (n=24) pig genotypes. Twenty-four boars from each genotype were selected and fed for six months on one of four diets containing selenium and zinc at high or low concentrations in a 2 × 2 × 2 factorial arrangements. Selenium concentrations in the diets were either low (0.26 mg/kg) or high (0.65 mg/kg) and zinc were either low (0.35 mg/kg) or high (0.74 mg/kg). The pigs were slaughtered after four months and testes were dissected, weighed and lengths, widths and circumferences measured. There were no increases in left and right testes weight indices in Kolbroek boars as selenium and zinc were increased ($P > 0.05$), while the indices decreased in LW × LR boars ($P < 0.05$). There was no effects of selenium and zinc supplementation on testicular and epididymis lengths and weights ($P > 0.05$) in both genotypes. There were no genotypes, selenium and zinc effects on seminiferous tubule area, density of spermatogonia, Sertoli nuclear volume and density of Leydig cells in the two genotypes ($P > 0.05$). There were no selenium and zinc effects on the germinal epithelium in Kolbroek boars ($P > 0.05$). In conclusion, the combination of selenium and zinc supplementation did not have an effect on accessory sex glands and spermatogenesis in both genotypes. Therefore, supplementation of selenium and zinc levels did not increase the weight of accessory sex glands, testicular and epididymis lengths and weights and spermatogenesis and seminiferous tubules.

Keywords: histomorphometry, minerals, indigenous pigs, crossbreed

7.1 Introduction

Selenium and zinc are required to increase performance and improve health and welfare in pigs (Close, 2003). Both zinc and selenium act as co-factors in the synthesis of anti-oxidants enzymes: superoxide dismutase and glutathione peroxidase (Bertelsmann *et al.*, 2007). Kolbroek boars are South African indigenous pigs with a tendency to put on excess fat, and are likely to require higher selenium levels especially for its anti-oxidative properties compared to the fast growing leaner genotypes. These pigs are more tolerant of various diseases and have a higher capacity to utilize fibrous and poor quality feed resources compared to exotic genotypes (Halimani *et al.*, 2010). The zinc requirements in rations for growing and finishing pigs lie between 50 and 100 ppm (Hill *et al*., 2000). The National Research Council's (NRC,1998) established selenium requirements for growing pigs as: 0.3 ppm for 3 to 10 kg pigs, 0.25 ppm for 10 to 20 kg pigs, and 0.15 ppm for 20 to 120 kg pigs. Therefore, there is need to evaluate the important of high and low inclusion levels of selenium and zinc LW × LR and Kolbroek pigs. Little information regarding high nutrient requirements and the need for intensive management systems make fast-growing pigs unsuitable for farming under harsh environmental conditions.

Toman *et al.* (2014) reported that both the testes and epididymis require an exogenous supply of selenium to synthesise a variety of selenoproteins (Shalini and Bansal, 2007) although their role in spermiogenesis and post-testicular spermatozoa maturation are not clearly defined (Toman *et al.*, 2014). Large White × Landrace boars'fed selenium supplementation had a greater number of Sertoli cells, spermatids and mature secondary spermatocytes (Marin-Guzman *et al.*, 2000). The effectsof dietary selenium supplementation on spermatogenesis in slow-growing Kolbroek boars has not been reported. Zinc deficiency resulted in underdevelopment of the Leydig cells, reduced sensitivity to luteinizing hormone (LH) and

impaired steroidogenesis which depletes testosterone production and inhibits spermatogenesis (Wilson, 2010). The effect of zinc supplementation in Kolbroek boars is also largely unknown. There is also no information on the interaction between selenium and zinc supplementation on testicular morphology, accessory sex glands and spermatogenesis in Kolbroek boars.

Testicular morphometric analyses are important for the description of the spermatogenic processes of each species, and all quantitative parameters relating to the seminiferous tubules have positive relationships with the spermatogenic activity (Nunes *et al*., 2017). Weight, length and width of testes are useful indicator traits for increased spermatozoa production and spermatogenic potential in pigs (Paula and Navarro, 2001; Valença *et al*., 2013).

Determining the effects of high and low inclusion levels of selenium and zinc supplementation is important to improve the productive system of genotypes. Therefore, the current study was designed to determine the interaction of selenium and zinc supplementation on testicular and accessory sex glands measurements, spermatogenesis and testicular histology in LW × LR and Kolbroek boars. It was hypothesised that there was no interaction of selenium and zinc on testicular development, histological morphology and spermatogenesis.

7.2 Materials and methods

7.2.1 Study site

The study was conducted at the Agricultural Research Council, Animal Production Institute (ARC-API), Irene, South Africa. The ARC-API campus is located at 25^0 34' 0" S and 28^0 12' 0" E and is located in the Highveld Centurion of South Africa and situated at an altitude of 1525 m above sea level. The average annual temperature is 18.7 °C.

7.2.2 Experimental pigs, housing and management

A total of 24 LW × LR boars with an average weight of 81.6 ± 1.7 kg body weight and 24 Kolbroek boars with an average weight of 74.2 ± 1.7 kg body weight were used in the study. All the boars were 7 to 8 months old. They were allowed to acclimatize for two weeks on the different diets and water was supplied *ad libitum*. The LW × LR and Kolbroek boars were each supplied with 2 kg feed mixture containing selenium and zinc in different proportions per day until the end of the experimental diet for six months. The pens were cleaned daily and the pigs were dipped in Triatix® (Amitraz) every two weeks against ectoparasites and were dewormed once a month against endo-parasites using Valbazen® (Albendazole). The pens for the LW × LR and Kolbroek boars measured 2 × 1.5 m in environmentally controlled houses with the temperature ranging from 22 to 25 ℃. The study was carried out in accordance with the recommendations in the Guide for the Care and Use of Animals under the guidelines of the Agricultural Research Council, Animal Production Institute Animal Ethics Committee (Reference: APIEC/16/002).

7.2.3 Experimental design and diets

Twenty-four Kolbroek and 24 LW × LR boars were used in a 2 × 2 × 2 (genotype × zinc × selenium concentration) factorial arrangement. Six pigs of each genotype were randomly assigned to four experimental diets containing either high or low levels of selenium and zinc. The four diets were: 1) low selenium (0.26 mg/kg) and low zinc (0.35 mg/kg) (LSLZ) boars was used; 2) high selenium (0.65 mg/kg) and high zinc (0.74 mg/kg) (HSHZ); 3) low selenium (0.26 mg/kg) and high zinc (0.74 mg/kg) (LSHZ); and 4) high selenium (0.65 mg/kg) and low zinc (0.35 mg/kg) (HSLZ) were used in this study.

A total mixed ration was formulated to supply14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg DM and 11.6 g lysine/kg which meet the requirements of growing pigs (NRC, 1998). The dry matter (DM), ash, crude protein (CP), ether extract (EE), zinc, selenium, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed following the procedures from the Association of Official Analytical Chemists (2005) and van Soest (1963). The ingredient and chemical composition of the diets are shown in Table 7.1.

7.2.4 Measurements of accessory sex glands and testes

At the end of the experimental period after six months all six boars from each treatment were humanely slaughtered for accessory sex glands and testicular morphometric measurements, such as spermatogenesis and histology analyses. The accessory sex glands and testes were removed, weighed and the testes lengths and widths were measured at the Germplasm Conservation and Reproductive Biotechnologies Section of the Agricultural Research Council, Irene, Pretoria, South Africa.

The weights of left and right testicles, seminal vesicles, prostate and bulbourethral glands of LW × LR and Kolbroek boars were measured using an electronic scale (VIbra Shinko DenshI Co., Ltd. Japan). Testes lengths and widths (cm) were measured using a Vernier callipers (Range: 0 - 150 mm 1/128, 6” 0-150 mm). The weights and lengths of the testes and accessory sex glands were expressed as a proportion of the final weight as indices to enable comparisons across the genotypes. Average lengths and diameters were used to calculate paired testicular volume, assuming each testicle had a prolate spherical shape. The formula used to determine paired testicular volume was:

Paired testicular volume = $4/3 \times \pi \times$ (average testis length) × (average testis diameter) (Sotos and Tokar, 2012).

Table 7.1: Ingredient and chemical composition of the diets

Ingredient (%)	Diets[1]			
	HSLZ	HSHZ	LSHZ	LSLZ
Yellow maize	62.25	62.25	62.25	62.25
Hominy chop	8.64	8.64	8.64	8.64
Feed lime	0.27	0.27	0.27	0.27
Monocalcium phosphate	2.95	2.95	2.95	2.95
Soya bean oil cake	24.69	24.69	24.69	24.69
Vitamin mineral premix[3]	0.20	0.20	0.20	0.20
Salt	1.00	1.00	1.00	1.00
Zinc	0.35	0.74	0.74	0.35
Selenium	0.65	0.65	0.26	0.26
Chemical composition analysis (%)				
Protein	16.74	16.74	16.17	16.72
Dry matter	90.45	90.65	90.28	90.22
Crude fibre	3.31	3.08	3.31	3.59
NDF	20.17	21.47	19.95	19.59
ADF	4.58	4.94	4.41	4.96
Digestible energy MJ/kg	20.31	20.71	20.44	20.21
Phosphorus	0.87	0.72	0.78	0.89
Calcium	0.80	0.60	0.76	0.68
Zinc	0.04	0.06	0.06	0.04
Selenium	0.006	0.006	0.001	0.001

[1]HSLZ = high selenium, low zinc; HSHZ = high selenium, high zinc; LSHZ = low selenium, high zinc and LSLZ = low selenium, low zinc

[2]NDF = neutral detergent fibre, ADF = acid detergent fibre

[3]The following minerals; 4.5 mg vitamin B2, 0.03 mg vitamin B12, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous.

7.2.5 Histology of testes

A portion of any parts of testes were cut immediately, fixed in 5 % formaldehyde and embedded in paraffin and set aside for morphometric analysis. After fixing, the samples were taken to the University of Pretoria Pathology Laboratory, where the fixed parenchyma samples were embedded in paraffin, cut into 5 µm thickness, mounted on microscope slides, and the sections were stained with Haematoxylin and Eosin (H and E) for analyses. Three slides were prepared for each sample.

7.2.6 Cell counting and spermatogenesis analyses

A total of 48 histology slides from the 24 LW × LR and 24 Kolbroek boars were analysed. Five sections were evaluated from each slide using 100X and 400X (10 X 40) magnification. The slides were covered with a microscopic cover slip and seminiferous tubule pictures were obtained using a camera (Olympus DP 70, magnification 40X, Olympus America Inc., Melville, NY) mounted on a photomicroscope (BX50, Olympus America Inc). Digital cameras were calibrated using a micrometer to facilitate measurements that were then made using Sigma Scan Pro 5 (SPSS Inc., Chicago, IL). Two diameter measurements were made and averaged for each of 100 round or almost round seminiferous tubules per slide, with the second measurement approximately 90° of the first. Ten round seminiferous cords/tubules and their epithelium heights were measured in the 5 µm tissue sections from the right and left testes at a final magnification of 400X.

7.2.7 Volume density of the testicular components

The testicular components (seminiferous cords/tubules and interstitium), tubular parameters and germ and somatic (Sertoli and Leydig cells) cells were obtained using a 441-point grid

placed in an eyepiece of the light microscope as described by Drumonda *et al.* (2011). Ten fields of slides were randomly selected per animal in the 5 μm tissue sections at 400X magnification. Leydig cells (100X magnification), Sertoli cells (400X magnification), seminiferous tubules (40X magnification) and thickness of germinal epithelium cycle (100X magnification), according to the tubular morphology system (Almeida *etal.,* 2006) were counted to assess spermatogenesis efficiency, as described by Melo *et al.* (2014). Ten cross-sections around seminiferous tubules were randomly selected per each animal at 1000X magnification. The number cell per cross section was corrected for section thickness (5 μm) and nucleus diameter. Then, spermatogonia, spermatocyte, spermatids and spermatozoa were detected using an ocular graticule fixed to the eyepiece of normal light microscope. For each samples, the numbers of spermatogenic cells from a total of five random areas and counted to obtain the numbers of spermatogenic cells and mean percentages of spermatogenic cells.

7.2.8 Statistical analyses

The interactions of selenium, zinc and genotype on testicular development, histological morphology and spermatogenesis were evaluated using SAS (1999). The GLM procedure was also used to determine the effect on interaction of selenium, zinc and genotypes. A 5% significance level was used. The model used to compare effects of genotype, selenium and zinc on testicular development, histological morphology and spermatogenesis was:

$Y_{ijkl} = \mu + S_i + Z_j + G_k + (S \times Z)_{ij} + (S \times G)_{ik} + (Z \times G)_{jk} + (S \times Z \times G)_{ijk} + E_{ijkl}$, where:

Y_{ijkl} =testicular development, histological morphology, spermatogenesis.

μ = was the overall mean common to all observations

S_i = selenium level (i = LS, HS)

Z_j = zinc level (j = LZ, HZ)

G_k = genotype (k = Kolbroek, Large White × Landrace)

$(S \times Z)_{ij}$=selenium ×zinc interaction

$(S \times G)_{ik}$ =selenium × genotype interaction

$(Z \times G)_{jk}$= the interaction of zinc × genotype

$(S \times Z \times G)_{ijk}$=selenium × zinc × genotype interaction

E_{ijkl}= residual error.

7.3 Results

7.1 Accessory reproductive glands

The effects of selenium and zinc supplementation on final weight, indices of bulbourethral glands, prostate glands and seminal vesicles in LW × LR and Kolbroek boars are shown in Table 7.2. The LW × LR pigs had higher final body weights than the Kolbroek pigs ($P<0.05$). The LW × LR pigs fed low selenium had higher final body weights ($P < 0.05$) than those fed high selenium irrespective of zinc levels and Kolbroek pigs fed high and low selenium had similar final body weights. Kolbroek pigs had higher bulbourethral gland and prostate gland indices than LW × LR pigs ($P < 0.05$). The LW × LR pigs fed low selenium had lower bulbourethral gland and prostate gland indices than those fed high selenium diets irrespective of the zinc levels ($P < 0.05$). Selenium and zinc supplementation had increased on bulbourethral gland and prostate gland indices in Kolbroek pigs ($P <0.05$). There was no genotype, selenium and zinc supplementation effects on the seminal vesicles index ($P >0.05$).

7.2 Testicular measurements

The effect of selenium and zinc supplementation on indices of testicular and epididymis lengths and weights in LW × LR and Kolbroek boars are shown in Table 7.3. There were genotype effects with the LW × LR boars having higher indices than the Kolbroek boars ($P < 0.05$).

There was an increase in the right epididymis index in LW × LR boars fed with higher selenium diet ($P < 0.05$). The Kolbroek boars had higher right epididymis weight index than LW × LR boars ($P < 0.05$). There was an increase in selenium and zinc supplementation, testicular and epididymis lengths and weights index also increased ($P<0.05$) in both genotypes. Selenium and zinc supplementation had no influence on right testis weight index in Kolbroek pigs ($P >0.05$).

There was genotype, selenium and zinc interactions on right and left testis weight indices ($P < 0.05$). The LW × LR pigs fed low zinc had higher right testis weight index than those fed high selenium diets irrespective of zinc levels ($P<0.05$). The right and left testis weight indices increased with selenium and zinc supplementation in Kolbroek pigs ($P<0.05$). The LW × LR pigs fed high selenium levels had higher left and right testis width indices than those fed low selenium diets irrespective of the zinc levels ($P < 0.05$). An increase in level of selenium and zinc lead to an increase in left testis width index in Kolbroek pigs ($P <0.05$). The supplementation selenium and zinc increased the right testis width index in both genotypes ($P <0.05$). The LW × LR pigs fed high selenium levels had higher left and right testis length index than those fed low selenium diets irrespective of the zinc levels ($P < 0.05$). There was a genotype effects on left testis length index ($P < 0.05$). The LW × LR pigs had increase on left testis length index than Kolbroek pigs ($P < 0.05$). Selenium and zinc supplementation had an effects on left and right testis length index ($P < 0.05$). Kolbroek pigs had increased right epididyms weight index than LW × LR pigs ($P < 0.05$). The supplementation of selenium and zinc inclusion levels increased, right epididyms weight indices also increased of both genotypes ($P <0.05$).

Table 7.2: Effect of selenium and zinc supplementation on indices of bulbourethral, prostate glands and seminal vesicles weights of Large White × Landrace (n = 24) and Kobroek (n = 24) boars

Genotype[2]	Inclusion levels[1]		Initial weight (kg)	Final weight (kg)	Accessory sex glands[3]		
	Selenium	Zinc			Bulbourethral glands index	Prostate glands index	Seminal vesicles index
LW × LR	High	High	44.4^{a}	86.4^{b}	0.57^{b}	0.46^{bc}	0.30
	High	Low	43.8^{a}	87.4^{b}	0.56^{b}	0.45^{bc}	0.29
	Low	High	44.5^{a}	93.8^{a}	0.52^{a}	0.43^{ab}	0.29
	Low	Low	45.2^{a}	93.2^{a}	0.52^{a}	0.41^{a}	0.28
Kolbroek	High	High	42.1^{ab}	75.4^{c}	0.59^{b}	0.48^{c}	0.27
	High	Low	40.1^{ab}	75.2^{c}	0.58^{b}	0.47^{c}	0.27
	Low	High	39.3^{b}	74.5^{c}	0.58^{b}	0.48^{c}	0.29
	Low	Low	43.2^{ab}	76.6^{c}	0.57^{b}	0.46^{c}	0.30
SEM			1.79	1.64	0.013	0.012	0.012
P values	Genotype		0.0123	<0.0001	0.0001	0.0001	0.237
	Selenium		0.7035	0.0054	0.0101	0.0234	0.634
	Zinc		0.6520	0.6379	0.4670	0.0953	0.696
	Genotype × selenium		0.8330	0.0097	0.0456	0.1255	0.138
	Genotype × zinc		0.7098	0.7599	0.9737	0.9098	0.388
	Selenium× zinc		0.1746	0.8816	0.7588	0.7295	0.857
	Genotype × selenium× zinc		0.3834	0.4098	0.8372	0.8492	0.633

[a,b]Values with different superscripts within columns differ (P<0.05)

[1]High selenium = 0.65 mg/kg, Low selenium = 0.26 mg/kg; High zinc = 0.74; Low zinc = 0.35 mg/kg; [2]LW × LR= Large White × Landrace; index = weight or length as a proportion of final body weight

[3]Bulbourethral glands index, prostate glands index, seminal vesicles index

Table 7.3: Effect of selenium and zinc supplementation on indices of testicular and epididymis lengths and weights in Large White × Landrace (n = 24) and Kolbroek (n = 24) boars

Genotype	Selenium	Zinc	Right testis weight index	Left testis weight index	Left testis width index	Right testis width index	Left testis length index	Right testis length index	Right epididymis weight index	Right epididymis length (cm)
LW × LR	High	High	0.35^{b}	0.38^{ab}	6.2^{bc}	6.1^{ab}	13.5^{ab}	14.1^{d}	0.13^{c}	28.0
	High	Low	0.36^{b}	0.37^{ab}	6.6^{c}	6.4^{b}	15.1^{b}	13.0^{bcd}	0.13^{c}	25.8
	Low	High	0.37^{ab}	0.38^{ab}	5.9^{abc}	5.8^{ab}	13.7^{ab}	13.7^{cd}	0.12^{b}	26.5
	Low	Low	0.46^{a}	0.49^{a}	6.1^{abc}	6.1^{ab}	14.0^{ab}	13.6^{cd}	0.12^{b}	27.3
Kolbroek	High	High	0.34^{b}	0.34^{b}	5.4^{ab}	5.1^{a}	13.3^{ab}	10.8^{b}	0.15^{a}	27.2
	High	Low	0.37^{ab}	0.38^{ab}	5.0^{a}	5.0^{a}	10.6^{c}	10.9^{ab}	0.15^{a}	27.5
	Low	High	0.35^{b}	0.36^{b}	5.3^{ab}	5.1^{a}	11.9^{ac}	9.9^{a}	0.15^{a}	27.7
	Low	Low	0.32^{b}	0.35^{b}	5.9^{abc}	5.7^{ab}	12.9^{abc}	11.6^{abc}	0.15^{a}	27.7
SEM			0.017	0.589	0.40	0.39	0.93	0.76	0.003	0.78
P-Values	Genotype		***	***	***	***	***	***	***	NS
	Selenium		NS	0.07	NS	NS	NS	NS	*	NS
	Zinc		*	*	NS	NS	NS	NS	NS	NS
	Se× Zinc		NS	NS	NS	NS	NS	NS	NS	NS
	Genotype ×Selenium		**	*	NS	NS	NS	NS	**	NS
	Genotype ×Zinc		0.07	NS	NS	NS	NS	NS	NS	NS
	Genotype ×Se×Zinc		**	**	NS	NS	0.0646	NS	NS	NS

[a,b]Values with different superscripts within columns differ (P<0.05); *** P <0.001, ** P <0.01, * P<0.05 and NS= not significant
Se = Selenium; High selenium = 0.65 mg/kg, Low selenium = 0.26 mg/kg; High zinc = 0.74 mg/kg; Low zinc = 0.35 mg/kg; LW × LR= Large White × Landrace; index = weight or length as a proportion of final weight;

7.3 Effect of genotype, selenium and zinc supplementation on histology and spermatogenic morphology

The effects of selenium and zinc supplementation on spermatogenesis and seminiferous tubules of LW x LR and Kolbroek boars are shown in Table 7.4. The number of seminiferous tubules were higher ($P < 0.05$) in LW × LR and Kolbroek boars, as inclusion levels of selenium increased. Kolbroek boars had higher number of seminiferous tubules than LW × LR boars ($P < 0.05$). Selenium and zinc had no effects on number of seminiferous tubules in Kolbroek pigs ($P >0.05$). There was no genotype, selenium and zinc effect on number of seminiferous tubules ($P >0.05$). The Sertoli nuclear volume tended to be lower in the low selenium low zinc diet in the LW x LR but was highest in the same diet in the Kolbroek boars ($P < 0.05$). Selenium and zinc supplementation had no effects on Sertoli nuclear volume of both genotypes ($P >0.05$). There was no genotype, selenium and zinc effects on seminiferous tubule area, density of spermatogonia and density of Leydig cells ($P >0.05$) in the two genotypes. There were no selenium and zinc effects on the germinal epithelium in Kolbroek boars ($P >0.05$). Low selenium and high zinc diet had the highest ($P < 0.05$) thickness of germinal epithelium in LW × LR boars.

Table 7.4: Effects of selenium and zinc supplementation on spermatogenesis and seminiferous tubules of Large White × Landrace (n = 24) and Kolbroek (n = 24) boars

	Inclusion levels		Parameters					
Genotype	Selenium	Zinc	Number of seminiferous tubules (per 0.5 mm^2)	Seminiferous tubule area ($10^2\mu m^2$)	Sertoli cell nuclear volume (μm^3)	Density of spermatogenic cells ($x10^6$)	Density of Leydig cells ($x10^9$)	Thickness of germinal epithelium ($10^4\mu m^2$)
LW×LR	High	High	31.3^{b}	29.6	5.6^{ab}	51.6	81.0	76.2^{a}
	High	Low	30.4^{ab}	29.8	5.5^{ab}	55.8	80.5	76.8^{a}
	Low	High	28.9^{a}	28.8	5.6^{a}	56.7	84.4	83.6^{b}
	Low	Low	28.9^{a}	29.2	5.0^{b}	48.4	83.2	77.1^{a}
Kolbroek	High	High	30.1^{ab}	27.9	5.5^{ab}	56.8	81.0	78.5^{ab}
	High	Low	29.9^{ab}	29.3	5.4^{ab}	56.8	81.2	75.0^{a}
	Low	High	30.3^{ab}	28.5	5.3^{ab}	52.3	81.6	73.1^{a}
	Low	Low	30.1^{ab}	28.9	5.7^{a}	57.2	80.9	76.0^{a}
SEM			0.738	0.849	0.198	3.947	1.521	2.012
P-Values	Genotype		NS	NS	NS	NS	NS	0.060
	Selenium		NS	NS	NS	NS	NS	NS
	Zinc		NS	NS	NS	NS	NS	NS
	Genotype× Se		*	NS	NS	NS	NS	*
	Genotype × Zinc		NS	NS	NS	NS	NS	NS
	Se×Zinc		NS	NS	NS	NS	NS	NS
	Genotype×Se × Zinc		NS	NS	0.0859	NS	NS	*

[a,b]Values with different superscripts within columns differ (P<0.05); *** P <0.001, ** P <0.01, * P<0.05 and NS= not significant
Se = Selenium; High selenium = 0.65 mg/kg, Low selenium = 0.26 mg/kg; High zinc = 0.74; Low zinc = 0.35 mg/kg; LW × LR= Large White × Landrace; index = weight or length as a proportion of final weight;

7.4 Discussion

The study hypothesised that testicular development, histological morphology and spermatogenesis were affected by inclusion levels of selenium and zinc supplementation in both genotypes. The LW × LR pigs had higher final body weights than the Kolbroek pigs, was expected. The LW × LR boars were expected to consume more feed than the Kolbroek boars because of their bigger body size and gut capacity (Thacker and Haq, 2009). Owing to the differences in final weight which could be attributed to the different genetics and dietary effects, indices of each organ were calculated as a proportion of total body mass to allow for comparisons. The implications of the finding that Kolbroek pigs had higher bulbourethral gland and prostate gland indices than the LW × LR are not clear. It could be due to the diets with higher levels did not supply sufficient nutrients to meet the requirements of the LW × LR pigs. Surai and Fisinin (2015) reported that the seminal vesicles, prostate, bulbourethral glands increased as levels of addition of supplementation selenium increased in the boar. Speight *et al.* (2012) reported that the increases in prostate, seminal vesicle, and bulbourethral gland in Yorkshire × Landrace boars supplememnted with increasing levels of selenium. Secretions from the prostate gland are responsible for flushing out urine and any bacteria in the tract before entry of spermatozoa into the urethra. The bulbourethral glands produce the gelatin plug which seals the large volume of ejaculate in the female uterus following natural service. No information available on the interaction of selenium and zinc prostate gland, spermatozoa and volume of ejaculate of LW × LR and Kolbroek boars. Therefore, there is need to determine the effect of selenium x zinc supplement on reproduction traits of LW × LR boars.

Unlike the Kolbroek boars, the LW × LR pigs responded to low selenium by having lower bulbourethral gland and prostate gland indices than those on high selenium diets irrespective of the zinc levels. Large White × Landrace and Kolbroek boars require low selenium diets to

increase the weight of bulbourethral gland and prostate gland indices. The findings that the selenium and zinc supplementation had an effect on prostate glands of both LW × LR boars. Selenium and zinc are great extent, utilizing for meeting normal range prostate glands. NRC, (1989) reported that selenium supplementation is required to ensure animals stay healthy and exhibit maximum growth and reproductive performance. Among the reproductive organs, the testis had the highest selenium concentration, which exceeded that of the prostate glands. Oldereid *et al*., 1998; Marin-Guzman *et al*., 2000; Speight *et al.* (2012) reported that the increasing selenium supplementation with increases prostate glands in pigs. The results that showed no influence of selenium and zinc inclusion levels on testicular development disagrees with in: humans where zinc supplementation increases prostate gland, seminal fluid, testicular and spermatogenesis (Murarka *et al*., 2015); and in rats where selenium supplementation increases reproductive organ weights, spermatogenesis counts and testicular morphology (Lek *et al*., 1996); and in rams increases selenium supplementation caused increases testes and accessory genital gland, seminal glands (Mahan and Parrett, 2013). The findings that showed the genotype, selenium and zinc supplementation had no effects on the seminal vesicles index was surprising given that the vesicular gland produces most of the seminal fluid, energy sources, buffers and ions including zinc. At high level of selenium, the seminal vesicles index increase and low level started decreasing, this suggest the low levels zinc are deficiency. It could be increased seminal vesicles due to a decrease in the ejaculation of semen (Gadella and Harrison. 2002). Surai and Fisinin, (2015) reported that increasing of inclusion levels of selenium, the seminal vesicles and testicles increased in pigs. In addition, there is no information on how does interaction of selenium × zinc influence the bulbourethral gland and prostate and seminal vesicles of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that future research should further explore these areas.

The genotype × selenium × zinc interactions on right and left testes weight indices were mainly due to the LW × LR having lower indices at high selenium concentrations whereas there were no changes in the Kolbroek. Generally, levels of right and left testes weight indices increase mainly due toaccounted for the presence of the increased amount of growing smooth endoplasmic reticulum destined for production of steroid hormones before the onset of puberty (Sarmaa and Devi, 2017). Therefore, LW × LR boars require low selenium to increase the weight of right and left testes weight indices. The right and left testes weight, width and length indices were generally higher in the LW × LR boars than in the Kolbroek. Similarly Ding *et al.* (2016) reported that Meishan boars had lower testes weights than Duroc at puberty. Testicular length and circumference are measures of testicular size which were reported to be significantly correlated with body weight (Bratte *et al.,* 1999). Ytournel *et al.,* (2014) reported that the boar testes size may be an indicator of the number of the Sertoli cells, as well as spermatozoa production. In addition, Hung and Johnson, (1996) reported that the increased size of testes can be used to improve the reproduction capacity of boars used for artificial insemination. Masenya *et al.* (2012) reported that Kolbroek boars had a lower semen volume compared to Large White boar and attributed it to the influence of body weight. In addition, there is no information on how does interaction of selenium × zinc influence the right and left testes weight indices of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that future research should further explore these areas.

The genotype × selenium interaction in the right epididymis weight index was due to the LW × LR having a higher index when fed high selenium than boars fed low selenium irrespective of the zinc concentrations. These findings simple mean that LW × LR boars require high

supplementation of selenium to increased weight of right epididymis weight index. Therefore, the increasing the levels of selenium and zinc had increased the right epididymis weight index of LW × LR boars. Increasing in right epididymis weight index and could potentially increase feed intake and palatability ofselenium and zinc diets. The right epididymis weight index in the Kolbroek on the other hand was not affected by selenium and zinc supplementation. The differences between two reports are difficult to explain. It could be that pigs adapted differently to the utilization of the different selenium and zinc diets. Therefore, it was possible that the supplementation of selenium and zinc inclusion in the diet did not influence testis weight and epididymis weight of Kolbroek boars. The Kolbroek pigs surprisingly had a higher right epididymis weight index than the LW × LR; the implications of which are not clear given that the LW × LR had higher testicular size indices. It could be Kolbroek pigs require high selenium and zinc to increased the weight right epididymis index, howerver LW × LR pigs require high selenium and zinc to increased the weight right epididymis index. The the epididymis, which has a key role in maturation of spermatozoa motility and fertilization capacity, is also responsible for spermatozoa storage and transport has value as an indicator of testicular function (Griffiths *et al.*, 2007). In addition, there is no information on how does interaction of selenium x zinc influence the weight of epididymis of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that future research should further explore these areas.

Kolbroek pigs had a higher number of seminiferous tubules than the LW × LR pigs are contrary to findings of Speight *et al.*, 2012) that number of seminiferous tubules fromYorkshire × Landrace boars were similar. The finding that the number of seminiferous tubules was not influence by selenium and zinc inclusion levels in LW × LR and Kolbroek pigs. Garcia-Gil *et al.* (2002). reported that the seminiferous tubules increased as levels of zinc supplememtation

increased in pigs. Zhou *et al.* (2009) reported that the increases in diameters of seminiferous tubule in pigs supplememntated with increasing levels of selenium. This could imply that selenium and zinc levels in Kolbroek may have been limiting in this study. An increasing the levels of selenium and zinc, the number of seminiferous tubules also increased in Kolbroek boars and low level of selenium and zinc started decreasing, it could be that the diets with higher selenium and zincare sufficient nutrients to meet the requirements of the Kolbroek boars. The finding indicated that testicular indices in LW × LR were higher than in Kolbroek and the fact that seminiferous tubules are an important testicular histometric trait directly related to testicular weight as reported by Valenca *et al*. (2013). Of note however is the finding that the number of seminiferous tubules in LW × LR boars responded positively to selenium supplementation unlike those in Kolbroek. In addition, there is no information on how does interaction of selenium × zinc influence the number of seminiferous tubules of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that future research should further explore these areas.

This provides scope to improve this parameter through nutritional intervention in LW × LR. The finding that the Leydig cell and Sertoli cells was not influence by selenium and zinc supplementation of LW × LR. and Kolbroek pigs. The Sertoli cells and Leydig cells were not affected by genotypes. Sorti cells increased as levels of zinc supplementation increased in pigs (Garcia-Gil *et al.*, 2002). Marin-Guzman *et al* (2000) and Surai and Fisinin (2015) reported that boars fed on dietary selenium supplementation had increased the number of Sertoli cells and spermatogenesis. Cheah and Yang (2011) reported that the Sertoli cells and Leydig cells increased as levels of selenium supplementation increased the in pigs. Martins *et al.* (2018) reported that boars fed dietary selenium supplementation caused increases the Sertoli cells and round spermatids and they also presented a greater number of secondary spermatocytes. Kumar

et al. (2006) also reported that addition of zinc supplementation caused increases in Sertoli cells in boars. Marin-Guzman *et al.* (2000a) and Ahsan *et al.* (2014) reported that the increases in Sertoli cells and Leydig cells in Landrace × Yorkshire pigs supplemented with increasing of inclusion of selenium and zinc. Goodarzi *et al.* (2017) reported that increases of zinc supplementation had increased the number of Leydig cells in pigs. In addition, there is no information on how does interaction of selenium × zinc influence the Leydig cell and Sertoli cells of Large White × Landrace and Kolbroek pigs. It is therefore, pertinent that future research should further explore these areas.

The germinal epithelium was not affected by selenium and zinc suppplementation in Large White x Landrace and Kolbroek pigs. The spermatogenesis and germinal epithelium were not affected by genotypes. Abdu (2008) reported that spermatogenesis and germinal epithelium increased as levels of zinc supplemenetation increased in pigs. Cheah and Yang (2011) reported that the increases in spermatogenesis in pigs supplemented with increasing inclusion levels of zinc. Spermatogenesis increased as levels of selenium suppplementation increased (Behne *et al*., 1996). Marin-Guzman *et al.* (2000a) reported that the increases in germinal epithelium in Landrace × Yorkshire × Duroc pigs supplemented with increasing levels of selenium. A previous study by Jana *et al.* (2008) found that the spermatogenesis and germinal epithelium increased as levels of zinc supplementation increased in pigs. There is no information is currently available on interaction of selenium × zinc influence the spermatogenesis and germinal epithelium of Large White × Landrace and Kolbroek pigs. Future research could focus on the selenium x zinc of spermatogenesis and germinal epithelium of Large White × Landrace and Kolbroek pigs.

7.5 Conclusions

The combination of selenium and zinc supplementation increased accessory sex glands, right and left testis indices and spermatogenesis and seminiferous tubes of both genotypes. Supplementation of selenium and zinc levels did not increase the weight of accessory sex glands, testicular and epididymis lengths and weights and seminiferous tubules.

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Chapter 8

General Discussion, Conclusions and Recommendations

8.1 General discussion

Selenium and zinc are trace elements that are important for normal reproductive function in males. They have, therefore drawn great interest of researchers to increase fertility. Zinc is commonly used in pig diets because a large number of natural feedstuffs are marginally zinc deficient. The study proposed that selenium and zinc supplementation would increase growth, semen quality, epididymal spermatozoa quality, seminal plasma constituents, testicular morphology, testosterone hormones and histology, carcass traits and visceral organs weight of slow-growing Kolbroek and crossbred pigs.

Chapter 3 tested the hypothesis that both selenium and zinc improves body weight, ADFI, ADG and FCR of LW×LR and Kolbroek pigs. The study found that selenium and zinc supplementation had no effects on growth performance in both genotypes. There was no selenium × zinc interaction on growth performance of both genotypes. The hypothesis was, therefore, rejected since there was no improvement in growth performance in both breeds.

Chapter 4 tested the hypothesis that supplementation of boar diets with selenium and zinc improves the sexual behavioural, semen ejaculation volume and testosterone concentration of LW × LR and Kolbroek boars. There was an effect of genotype on, time of mounting without penis exposed (TMNP) and duration of ejaculaion. Kolbroek boars took more time than LW × LR to mount and ejaculate semen. The duration of ejaculation increased when LW × LR boars fed on LSLZ diets. There was on increase in semen volume and testosterone levels when the LW × LR boars were fed on HSHZ and HSLZ diets. Dietary inclusion levels of selenium and

zinc had an effects on sexual behaviour, ejaculation semen volume and testosterone concentration of both LW × LR and Kolbroek boars. The hypothesis was not rejected.

Chapter 5 tested the hypothesis that there was interaction of selenium and zinc supplementation on visceral organs, drip loss and carcass traits of LW × LR and Kolbroek pigs. The study observed no effects of selenium and zinc supplementation on visceral organs weight and carcass traits and primal pork cuts in both genotypes. There were effects of genotypes on weight of large intestine and small intestines, warm carcass weight, cold carcass weight, carcass length, drip loss, hindquarter circumference, length and weight and backfat thickness. The hypothesis was therefore rejected. It was noted, however, that there were no negative effects on carcass measures in the Kolbroek pigs.

Chapter 6 tested the hypothesis that selenium and zinc supplementation improves the epididymis. Genotype had no effects on macroscopic semen quality of both genotypes. Dietary levels of selenium and zinc had no effects on semen volume and spermatozoa concentration of both genotypes. Spermatozoa abnormalities were affected by selenium and zinc supplementation. Total motility, spermatozoa counts, spermatozoa velocity and amplitude of lateral head were not influenced by selenium and zinc supplementation. Dietary supplementation selenium and zinc had no effets on seminal plasma protein concentrations of zinc, potassium and calcium in the seminal plasma by in both genotypes. However, sodium, selenium, magnesium and phosphorus concentrations of seminal plasma were affected by the inclusion levels of selenium and zinc in diets. The dietary levels of selenium and zinc supplementation influenced Lipid peroxidation of both genotypes.

Chapter 7 tested hypothesis that there was no interaction of selenium and zinc on testicular development, histological morphology and spermatogenesis. There was no increased in left and right testes weight indices in Kolbroek boars as the inclusion levels of selenium and zinc increased, the indices decreased in LW × LR boars. The study observed that supplementation of selenium and zinc had no effects on the lengths and weights of testes and epididymis in both LW × LR and Kolbroek boars. There was no effect of genotype, selenium and zinc on seminiferous tubule area, density of spermatogonia, Sertoli nuclear volume and density of Leydig cells. Testicular development not affected by inclusion levels of selenium and zinc in both genotypes.

8.2 Conclusions

Supplementation of selenium and zinc have no effect on growth performance of both LW × LR and Kolbroek boars. There were selenium and zinc effect on sexual behaviour, testosterone concentration of both LW × LR and Kolbroek boars. Therefore, the increasing the inclusion levels of selenium and zinc, as increased the weight of visceral organ and carcass traits and primal pork cuts in boths LW × LR and Kolbroek boars. Supplementation of selenium and zinc had no effect on semen volume and spermatozoa concentration, total motility, total spermatozoa count, spermatozoa velocity and amplitude of lateral head, seminal plasma protein concentrations of zinc, potassium and calcium in the seminal plasma, lengths and weights of testes and epididymis and HOST+ and HOST- of both LW × LR and Kolbroek boars.

8.3 Recommendations and further research

The study proved that there is no need to increase inclusion levels of selenium and zinc above low selenium contains, 0.26 mg/kg low zinc 0.35 mg/kg, since all levels of selenium and zinc

have similar effects on the growth performance, sexual behaviour, testosterone concentration, carcass characteristics, epididymal semen quality, seminal plasma constituent and lipid peroxidation and testicular morphology and related endocrine. It is of advantage for small scale farmer because low selenium 0.26 mg/kg low zinc 0.35 mg/kg diet might be considering than other diet which is high levels. These findings suggest that the effects of selenium and zinc supplementation differ with genotypes. These minerals, however, produce an overall improvement in reproductivepigs performance. Determination of the exact level at which selenium and zinc should be included in Kolbroek boar diets would be highly beneficial in improving the reproductive status of breeding sire, maintain genotype diversity, improve the livehood of low resource farmers with a concomitant economic benefits to the pig industry. It is important to highlight that the adding of selenium and zinc requires careful consideration of likely imbalances of other mineral and nutritients, which may be harmful to pigs or may reduce profitability of the pig enterprises.

There is need for further studies to investigate the following:

- determine influence of increasing levels of selenium and zinc beyong the levels used in the current study on performance and boar fertility
- assess semen quality, epididymal spermatozoa quality, seminal plasma constituents, testicular morphology, testosterone hormonesand histology, carcass traits and visceral organ weights in different breeds and genotypes.
- determine optimum inclusion levels of selenium and zinc supplementation
- investigate the mechanism in which interaction of selenium and zinc act
- determine the effectiveness of selenium, zinc and α-tocopherol on libido of boars
- assess the physiological effect of selenium and zinc on spermatozoa motility,

- unravel selenium and zinc supplementation influences on thegenomic control of membrane damage