PROTEIN ACCRETION AND ITS EFFECTS ON GROWTH RATE AND TESTICULAR TRAITS OF KOLBROEK BOARS

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DECLARATION AND COPYRIGHT

I, Thivhilaheli Richard Netshirovha, student number **212087827**, declare that the dissertation: *Protein accretion and its effects on growth rate and testicular traits of Kolbroek boars*, submitted to the Central University of Technology, Free State for the Magister Technologies: AGRICULTURE is my own independent work and that all the sources used and quoted have been acknowledged by means of complete references; and complies with the code of academic integrity, as well as other relevant policies, procedures, rules and regulation of the Central University of Technology; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification. I also disclaim the *copyright* of this dissertation in favour of the Central University of Technology, Free State.

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Thivhilaheli R Netshirovha **DATE**

DEDICATION

I dedicate this study to the Netshirovha's family and staff of Germplasm Conservation & Reproductive Biotechnologies at the Agricultural Research Council (ARC), Irene, for their esteemed contributions that enriched my experience and knowledge during the course of this study.

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LIST OF ACRINYMS AND ABBREVIATIONS

ABBREVIATIONS	DESCRIPTION
AA	Amino acid
ADG	Average daily gain
ADTFI	Average daily total feed intake
ARC	Agricultural Research Council
BPG	Brain pituitary gonad
BW	Body weight
Ca	Calcium
CCW	Cold carcass weight
CL	Carcass length
cm	Centimeter
CP	Crude protein
DE	Digestibility energy
DM	Dry matter
DSP	Daily sperm cells
EBW	Empty body weight

EMA	Eye muscle area
EMF	Eye muscle fat
ETFM	elapsed time to fist month
FAD	Fractional accretion degradation
FAR	Fraction accretion rate
FCR	Feed conversion ratio
FSH	Follicle-stimulating hormone
g/d	grams per day
HI	Heat increment
HP	Heat production
IGF-1	Insulin-like growth factor 1
IPI	Ideal protein intake
IW	Initial weight
Kg	Kilogram
LEA	Loin eye area
LEL	Left epididymis length
LEW	Left epididymis weight
LH	Luteinizing hormone

LT	left testis
LTL	Left testis length
LTW	Left testis width
LTW	Left testis weight
LW	Live weight
MAX	Maximum
Min	Minimum
MJ	Mega joule
N	Nitrogen
NM	Number of mounts
NRC	National Research Council
^{o}C	Degree Celsius
OMD	Organic matter digestibility
P	Phosphorus
P^2	Back fat
PA	Protein accretion
PB	Whole body protein mass
PR	Protein retention

REL	Right epididymis length
REW	Right epididymis weight
RT	Right testis
RTL	Right testis length
RTW	Right testis width
RTW	Right testis weight
SC	Sertoli cell
TAGS	Time anongenital sniffing
TL	Testis length
TMNP	time mounts with penis exposed
TMWP	Time mount without penis exposed
TNS	Time nosing on dummy sows
TV	Testicular volume
TW	Testis width
vs	Versus
WCW	Warm carcass weight

GENERAL ABSTRACT OF THE STUDY

The objectives of the study were to evaluate the growth performance, age at the attainment of puberty, libido status, testicular morphology, carcass quality, nitrogen balance and protein accretion in Kolbroek boars fed diets that contained different levels of protein. At the age of 3 months old on live weight of ±14 kg, boars were randomly allocated to three protein diets: Diet 1 (n=5); 2 (n=4) and 3 (n=5) comprising of 10, 13 and 16% protein; respectively. Growth performance, attainment of puberty, testicular morphology, carcass quality, and nitrogen balance were measured from boars fed three protein diets groups. There was no significant effect of the dietary protein on growth performance of Kolbroek boars. However, there was a positive correlation between feed intake and average daily gain (r= 0.78). High negative correlations were recorded between feed conversion ratio and average daily gain (r= -0.94), feed intake and feed conversion ratio (r= -0.57). Moreover, there was a marginal negative correlations between feed intake and back fat thickness (r= -0.08) and between feed conversion ratio and back fat thickness (r= -0.02). Gonadal measurements and hormonal concentrations were not significantly affected by different concentration of protein inclusions. However, there was a significant difference between 13 (3.5 mm) and 16% (5.0mm) protein diet on the right testicle width. Additionally, there was a positive correlation between left testis length and right testis length (r=0.90). The left and right epididymis weight also showed positive correlations. The visceral organs, water-holding capacity and carcass quality were not affected by the different protein levels. No significant difference was observed on nutrient digestibility and nitrogen balance, regardless of protein diets. In conclusion, there was no effect of dietary protein levels on growth performance, age of attainment of puberty, libido status, testicular morphology, carcass quality, nitrogen balance and protein accretion of Kolbroek boars. However, protein diet affected the weight of epididymis and influence the differences between left and right testicle.

Key words: *Kolbroek boars, Protein diet, growth performance, puberty, testicular morphology, carcass quality*

Chapter 1

CHAPTER 1: BACKGROUND OF THE STUDY

1.1 GENERAL INTRODUCTION

Kolbroek is a South African indigenous pig breed that possesses superior genetic traits such as the ability to survive by scavenging outside homesteads. In addition, these pigs do well on a high fibre diet and have high disease tolerance and a docile nature (Swart *et al.,* 2010). Kolbroek pigs have also been demonstrated to be more tolerant or less susceptible to internal parasites, such as *Ascaris suum* (Chimonyo *et al*., 2010). However, they are regarded as less efficient than the exotic pig breeds, such as Landrace and Large White. This is due to the fact that they tend to put on excess fat which is seen as a disadvantage (Ramsay *et al.,* 1994). Information on the nutritional requirements of Kolbroek pigs is scarce and inadequate (Umesiobi & Iloeje, 1999). Boars contribute higher genetic progress in pig herd (Umesiobi & Iloeje, 1999; Umesiobi, 2009a, Umesiobi, 2009b, Umesiobi, 2010a); hence there is a need to elevate the growth and reproductive performance of the Kolbroek boars by placing them on feeds that contain different protein levels.

Indigenous boars received little attention in a review by National Research Council (NRC, 1998). It was estimated that a boar requires a 2 kg feed intake per day of a 13% crude protein diet (lysine 0.6% and total sulphur amino acids 0.42%) containing 13.66 MJ (mega joule) Digestibility Energy/kilogram. However, this was formulated specifically for exotic pig breeds. When evaluating nutritional effects on boars, the following categories should be considered: quality and quantity of sperm (Kemp & Nicoline, 2001), libido and fertility of the sperm (Umesiobi, 2000), welfare and environmental impacts (Wilson *et al*., 2004).

In growing pigs, protein accretion is a major component of total amino acid. The rate of protein accretion increases from birth to a maximum and then declines gradually to almost zero as maturity is reached (Torrallardona *et al*., 1994). Maximum protein accretion is reached at variable age and body weight depending on sex and genetics of the pig breed. Dietary protein restriction during the growing period has negative effects on weight, age at puberty and sexual development (Kemp & Nicoline, 2001). However, information regarding nutritional requirements and effects of nutrition on reproductive aspect of Kolbroek boar is relatively scarce.

Indigenous boars such as Large White reach puberty between 6.5 and 7 months with an average body weight that ranges between 61-106 kg (Foote, 1994). In contrast, the onset of puberty in other indigenous pig breeds occurs between 4 months of age (Campell *et al*., 1990; Chimonyo & Dzama, 2010, Trudeau *et al.,* 1992). Moreover, libido and semen quality in boars can be adversely affected if protein and energy intake are reduced (Day, 2000). Protein content of less than 12% has been shown to significantly reduce boar interest in mounting a dummy sow and ejaculating (Whitney *et al*., 1999). Therefore, there is need to evaluate the effect of protein supplementation on sexual behaviour of Kolbroek boars.

Balanced diet for boars is important for sexual development and mating performance at puberty. It is known that variation in nutrition can be tolerated by mature boars without any detrimental effect on sperm quality (Umesiobi, 2009; Ford & Wise, 2011). Optimal semen production is important for assisted reproductive technologies in pig breeding programs (Umesiobi, 2010a, Umesiobi, 2010b), such as artificial insemination (AI) and *in vitro* embryo production (IVEP). There is little information available about the effects of protein accretion on testicular morphology, growth rate and sexual behavior on South African Kolbroek boars.

The semen volume produced by the spermatogenetic tissue increased with age, body weight and testis weight in boars (Ugwu *et al*., 2009). This indicates that boars with higher body and testis weight may produce more sperm. There is the need to match body weight, testis size and sperm production capacity in selecting boars for improved reproductive efficiency (Ugwu *et al*., 2009). It has been shown that the epididymis and accessory sex glands also play a role for the functional status of male gametes (Elzanaty *et al*., 2002). However, there is little information on

the leydig cell cytoarchitecture organization in the testis, its volume density and individual size (de Almeida *et al*., 2006). There is also little information reported on the testis structure and functioning membrane integrity, particularly related to spermatogenesis in Kolbroek boars.

1. 2 PROBLEM STATEMENT

There is limited published information on the standardised protein requirement for the South African indigenous Kolbroek pigs breed (Chimonyo *et al*., 2005). Furthermore, there is a lack of information regarding the effects of protein accretion on growth rate, puberty, sexual behaviour and testicular morphology of indigenous Kolbroek boars. Interestingly, it is known that the major determinant of the reproductive performance of boars is the growth pattern and ultimate size of the testis (Ugwu *et al*., 2009). In view of the aforementioned paucity of information on nutritional requirements of Kolbroek boars; , there is a need to compare the body weight and testis size in selecting boars for improved reproductive performance of Kolbroek boars. Back fat thickness of exotic pigs have been intensively studied (Babu, *et al*., 2004), however little information is available on back fat thickness in Kolbroek boars.

Few studies have been done about the age of puberty in indigenous Kolbroek boars in relation to their protein accretion levels. Furthermore, very little information contrary to commercial pig breeds is available on effect of protein on meat products from Kolbroek pigs. Indigenous pig breeds also appear to adapt in difficult or specific environments and handling conditions which may influence the meat quality (Hoffman *et al*., 2005).

1.3 MOTIVATION FOR THE STUDY

Indigenous pigs were long regarded as unsuitable for intensive commercial breeding program, mostly because of their slow growth and inadequate meat production (Prolit, 2004). Nevertheless, many indigenous African breed populations exhibit well established adaptations to prevailing environmental and management conditions. This represents a valuable genetic resource for improving the genetic conservation and diversity of pig breeds and for sustainability of animal production systems. Indigenous populations are known to add potential

characteristics towards genetic variation, which invariably contributes new diversity for the improvement of commercial lines (Blott *et al*., 2003). Therefore, there is need to evaluate the possibility of using South African indigenous pigs breeds for production of pork for fresh consumption (Hoffman *et al*., 2005).

Protein intake on reproduction of pigs has been opposite and focused on the effects of very high protein and amino acids intakes (Louis *et al.,* 1994). There is little information about the age of puberty, libido, body condition and growth rate, testicular morphology in South African indigenous Kolbroek boars.

1.4 OBJECTIVES:

1.4.1 PRIMARY OBJECTIVES

- The primary objective of this study was to evaluate the effects of protein accretion on growth rate and testicular traits of Kolbroek boars.

1.4.2 SPECIFIC OBJECTIVES

The specific objectives of the study are to:

- determine the effect of dietary protein concentration on growth rate in the South African indigenous Kolbroek boars.
- evaluate the effect of dietary protein concentration on age at the attainment of puberty in Kolbroek boar.
- ascertain effects of different dietary protein concentration on testicular morphology in the South African indigenous Kolbroek boars.
- analyse the effect of feeding different levels of dietary protein on nutritional digestibility and carcass quality of the South African indigenous Kolbroek boars.

1.4.3 HYPOTHESIS

- A 10 % dietary protein concentration will improve growth rate, carcass quality and protein digestibility of the South African indigenous Kolbroek boars.

- Kolbroek boars fed 13 and 16% dietary protein, will attain the age of puberty early with subsequent improvements on testicular morphology and sperm quality in the South African indigenous Kolbroek boars.

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Chapter 2

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Kolbroek is a South African indigenous pig breed that possesses valuable traits such as diseases tolerance and adaptability to harsh environmental conditions (Masenya *et al.,* 2011). Indigenous pigs are small sized, weighing approximately 0.5 kg at birth and 45.5 kg at one year of age (Anugwa & Okwori, 2008). Indigenous pigs have been recommended as an alternative source of cheap, high quality animal protein that suits escalating human population (Ironkwe & Amefule, 2008). Moreover, they are a major source of livelihood for many communities worldwide (Halimani *et al.,* 2010), particularly the resource-poor smallholder farmers whose majority are found in developing countries. However, religion also play role in consumption of pork in Africa.

Figure 2.1: Kolbroek boar (ARC-API PIG)

2.2 Protein levels on the growth performance of kolbroek boars

2.2.1 Body weight and growth rate

Slow growth rate is an inherent setback in the use of the indigenous pigs for commercial production. Nevertheless, the purported slow growth syndrome attributed to the indigenous pigs can be an advantage during adverse conditions associated with feed shortages commonly noted in communal production systems. According to Kanengoni *et al.* (2004), the Zimbabwean indigenous Mukota pigs exhibit relatively low growth rates of 360 g/day compared to 660 g/day for Large White pigs under restricted feeding regimen. The indigenous pigs show a remarkable peak in growth between 12 and 14 weeks post-weaning (Kanengoni *et al*., 2004), associated with early maturation, resulting in early fat deposition than the fast growing imported pigs (Chimonyo *et al*., 2005). Mukota pigs reach slaughter weight of 35 to 40 kg at six months of age, in contrast to Large White pigs which reach slaughter weight of 100 kg at a similar age (Chimonyo *et al*., 2005). Hence, there is a need to evaluate the effect of protein on growth performance of Kolbroek boars.

2.2.2 Feed intake

Residual feed intake is merely the difference between the expected intake of the pigs and what they actually consume. Dietary protein is one of the most important components in pig production, as it promotes production efficiency and quality of muscle, thereby affording the most desirable and economically valuable body tissue (Bünger *et al*., 2012). The rate of feed intake is generally associated with the pig's attempts to maintain dry energy intake (Adesehinwa, 2008). Pigs will compensate for decrease or increase in the nutrient density of the diet by increasing or decreasing their feed intake (Adesehinwa, 2008). Therefore, there is need to evaluate the effect of protein on the feed conversion ratio in Kolbroek boars.

2.2.3 Feed conversion ratio

Feed Conversion Ratio, or feed conversion efficiency (FCE), is the ability of livestock to turn feed mass into body mass. According to Anugwa & Okwori, (2008), pigs gained more weights and had better feed conversion ratios on 16% than on the 12% dietary protein. According to Adesehinwa (2008), there was no significant effect of protein inclusion levels on feed efficiency in boars fed barley-soybean meal diets containing either 17 or 13% protein from 50 to 88.6 kg in Large white x Landrace x Duroc. These observations warrant the quest to evaluate the effects of dietary protein levels on average daily gain for the Kolbroek boars.

2.2.4 Average daily gain

The weight of a pig is an important indicator of its growth, health and readiness to go to the market (Wang *et al*., 2008). Pigs fed 16% dietary protein level gained more than those on 10 and 13% dietary protein levels (Chen *et al*., 1995). Additionally, at higher levels of lysine, pigs fed 16% dietary protein gained more weight than those fed 12% dietary protein (Louis *et al.,* 1994). The pigs on diet supplemented with 100g/100kg diet had the best result in terms of daily weight gains, protein efficiency and feed conversion ratio (Adesehinwa *et al*., 2008). However published research is very limited on the optimum dietary crude protein inclusion levels for growing boars in relation to the growth performance characteristics.

2.3 ATTAINMENT OF SEXUAL MATURITY IN BOARS

2.3.1 Puberty and libido in indigenous boars

Puberty is the time of first appearance of sperm in the seminiferous tubules, it occurs in the exotic breeds of boars at an average age of 5 to 6 months and in the indigenous boars at about 4 months (Okwun *et al*., 1996). According to Young *et al.* (1990), if the attainment of puberty was dependent on achieving a certain threshold of a particular body component, variation of this component around the time of puberty would not be normally distributed. The onset of puberty, and therefore the ensuing rise in steroid genesis, is highly variable between individual

boars and is influenced by factors such as nutritional status (Brown, 1994), stress and social rank (de Jonge *et al*., 1996), and breed (Babol *et al*., 2004). There is therefore, a need to evaluate the age and body weight at which puberty is attained in South African indigenous Kolbroek pigs.

Libido or sexual desire exemplified by reaction time (Umesiobi & Iloeje, 1999), is an important aspect of male reproductive function. It may be impaired by mismanagement of young boars during service (Hafez, 2000). Boars with high testosterone also have high libido (Flowers, 2008). In boars, LH and FSH stimulate spermatogenesis and secretion of testicular steroids responsible for maintaining libido and controlling gonadotropin release *via* classical negative feedback mechanisms (Estienne *et al*., 2009). Hence there is need to evaluate the effect of protein on the age of puberty and libido on indigenous Kolbroek boars.

2.3.2 The male sex hormone testosterone

Gonadotropin releasing hormone (GnRH) is important because it is responsible for inducing the release of FSH (Follicle Stimulating Hormone) and LH (luteinizing hormone) from the pituitary gland, which is located just below the hypothalamus (Knox, 2003). These hormones are responsible for regulating testicular function (Hess & Franca, 2008). Secretion of LH and FSH stimulates spermatogenesis and the testicular secretion of testosterone and estrodiol, two steroid hormones that together are responsible for maintenance of libido (Estienne *et al*., 2000). Testosterone has a variety of important functions in spermatogenesis and male sexual behaviour (Hess & Franca, 2008). Luteinizing hormone released from the anterior pituitary gland stimulates production of androgens from the leydig cells (Trudeau *et al*., 1992; Ogbuewu *et al*., 2007). Several hormones, including luteinizing hormones and follicle stimulating hormones, are released into the blood stream from the pituitary gland, a garden pea-sized organ that is located just below the brain (Wilson, 2004).

2.3.4 Interaction of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)

FSH stimulation hormones of the testes start the process of sperm production (spermatogenesis) by initiating sperm cell division and development (Knox, 2003). Moreover, it influences the sexual behaviour and testicular function of the boar. The level FSH in boars is necessary to maintaining testis and also to regulation of FSH secretion (Hess & Franca, 2008). FSH concentrations or measurements of sperm production of testosterone levels in the endocrine feedback regulation system (Walker *et al.,* 2004). Follicle-stimulating hormone stimulates the sertoli cells to produce androgen-binding proteins; change testosterone to dihydro-testosterone and estrogen; and secrete inhibiter (Wagner & Claus, 2009). Sertoli cell maturation, lumen formation occurs within seminiferous tubules and germ cells proliferate rapidly followed by onset of sperm (McCoard *et al*., 2003). The sertoli cell division stops before puberty, with the sertoli cell population becoming stable thereafter, mostly because sertoli cells can support only a limited number of germ cells (Hess & Franca, 2008). The increase in FSH secretion that occurs after unilateral castration has minimal effect on sertoli cell proliferation (McCoard *et al*., 2003; Umesiobi, 2010).

LH and maternal CG (chorionic gonadotropin) have been implicated in the regulation of Leydig cell development in several species such as pigs. These hormones cause a large increase in intratesticular testosterone followed shortly by increased serum levels of testosterone in the foetus (Knox, 2003). LH stimulates aromatase activity and androgen secretion of pigs leydig cells (Hafz, 2000). In the boar and bull, plasma LH levels increase until testosterone begins to control LH secretion (McCoard *et al*., 2003).

2.4 FACTORS AFFECTING PUBERTY IN PIGS

2.4.1 Nutrition

Nutrition is the primary exogenous element of the form this line tissue and organs, and the related to attainment of sexual maturity (Coetzee & Casey. 2009). In the pig, nutrition provides essential amino acids necessary for the normal body function in the amounts and essential for

the particular pig need (Adesehinwa, 2008). Report by Kerr & Cameron (1998) suggests that malnutrition resulting from inadequate, excess or imbalanced nutrients intake may delay puberty.

Protein intake may affect sperm cells by altering hormone production and secretion in boar (Wu, 2009). Boars with a low protein intake took longer to start ejaculating and remained on these semen collection dummy for a shorter time (Louis *et al.,* 1994). High levels of amino acids may partly mediate the effect of high-protein intake on circulating concentrations of hormones in animals (Wu, 2009). Overfeeding and excess body weight also contribute to the increased incidence of feet and leg problems as well as the decrease of libido in boars (Wilson *et al*., 2004).

2.4.2 Environment

A number of studies have been reported concerning the genetic and environmental factors influencing age and weight at puberty (Evans & O'Doherty, 2001). Photoperiod has minimal influence on the timing of puberty (Anderson *et al.,* 2000). Heat stress, on the other hand, may have a detrimental effect on boar sperm production and semen quality. According to Paterson *et al* (1990) & Andersson *et al*. (1998), photoperiod has significant effects on sexual maturation of young crossbred domestic boars. The variability of age at puberty of gilts and to other aspects of the environment in which they are kept has also been reported (Evans & O'Doherty, 2001).

2.4.3 Season

In boars, seasonal changes are influenced by photoperiod and temperature (Cheon *et al*., 2002). Heat stress may contribute to the delay of puberty during summer (Flowers, 2008), but the long light duration probably plays a more important role, seasonal variations can be repeated under constant temperature by altering light patterns to mimic natural photoperiods (Paterson & Pearce, 1990). Animals reared under an autumn-winter light had higher plasma concentrations of testosterone, heavier bulbourethral glands and a considerably higher

frequency of animals with a mature spermatogenesis at slaughter at 150 days of age than animals reared under a spring-summer photoperiod (Andersson *et al.,* 1998).

2.4.4 Breeds

Variations in exotic breeds and in management regimens, influence age at puberty (Paterson & Pearce, 1990). Therefore, breed influences age at puberty, but, among commercial breeds, management factors can have a greater effect than breed on the onset of reproductive capability (Evans & Doherty, 2001).

2.4.5 Age and weight

According to Evans & O'Doherty (2001), age is one of the most important factors determining puberty attainment. It was suggested that live weight on its own does not account for the induction of puberty, but contributes as one of the factors leading to puberty (Gaughan *et al*., 1997). Live weight, backfat thickness and average daily gain, also affect the attainment of puberty in pigs (Evans & Doherty, 2001). According to Beltranena *et al.* (1993) reported that an indicator of attainment of puberty before the first mating and the back fat thickness should be a minimum of 6 mm.

2.4.6 Genetic factors

The response of breeding animals to low backfat deposition is directly linked to the animal genotype (Gaughan *et al*., 1997). Animals that are selected for lower backfat thickness appear to grow bigger (Gaughan *et al*., 1997). Genetic changes in body composition and appetites are associated with reduced reproductive performance (Kerr & Cameron, 1998). Hence, body fat and muscle content are associated with age at puberty, genotype levels of body fat and muscle required for the onset of puberty to occur (Kerr & Cameron, 1998). Genetic changes in body composition and appetites are associated with reduced reproductive performance in contemporary comparisons of lines of pigs selected for different component of efficient lean growth (Ker & Cameron, 1998).

2.5 TESTICULAR MORPHOLOGICAL OVERVIEW OF BOAR TESTIS

2.5.1 The scrotum

The areas of scrotum in contact with the caput and caudal epididymis are small in comparison with the covering the testis and epididymis temperatures exceeded testicular temperature at 23 and 34° C (Hafez, 2000). Therefore, the role of the scrotum is to regulate testes temperature to be (20°C) lower than body temperature (Knox, 2003). Muscle called the cremater, is found in the spermatic cord and contracts or body in cold weather or let them hang further away in hot weather (Knox, 2003). In the boar, the scrotum is less pendulous and sweating is less efficient (Hafez, 2000). Scrotum circumference supports on limited number of scrotum circumference established before puberty determines the rate of sperm production in sexually mature boars (Gnessi *et al*., 1997).

2.5.2 The testis

Testis is a complex organ that serves two crucial functions, vi &ii) synthesis and secretion of testosterone and ii) production of a sufficient number of competent sperm cells to attain fertility (Gnessi *et al.,* 1997). Cryptorchidism is the most frequent male sexual disorder in mammals, arising from a failure in the descent into the scrotum of one testis or both testes (Pinart *et al.,* 2001). In boars, the lamina propria plays an essential role in the maintenance of structural cohesion of the seminiferous tubes and is a constituent of the blood-testis barrier (Pinart *et al*., 2001). The basal lamina, surrounded by 2 layers of peri-tubular cells are separated by a fibrous layer (Gnessi *et al.,* 1997). Boars with larger testes had altered hypothalamic control of hypophyseal synthesis and release of Luteinizing Hormone (Pinart *et al.*, 2001). They are pulled through an opening called the inguinal canal, which allows passage of the testes and formation of spermatic cord.

2.5.3 Epididymis

The epididymis is under the control of testosterone prenatally and its growth increases around the time of puberty (Walker *et al*., 2004). Sperm storage is within the epididymis (Walker *et al.,* 2004), and the epididymis is an extremely large convoluted structure, which is closely attached to the dorsal part of the lateral surface of the testicle. Its functions include storage, maturation and absorption of sperm (Matthew & Oniovosa, 2005). In addition to the primary regulation of luminal fluid and iron transport, estrogen is also responsible for maintaining a differentiated epithelial morphology through a mechanism remaining to be discovered (Hess & Franca, 2008). In the epididymis, peristaltic activity of smooth muscle is large responsible for sperm transport. Sperm are transported through the epididymis in about 12 days in the boar (Hafez, 2000). Sperm in the tail of the epididymis are 2 to 10 more fertile than sperm from caput, apparently due to abnormalities in locomotion of the sperm taken from the head of the epididymis (Matthew & Oniovosa, 2005). The epididymis duct is a long, highly coiled duct. Its total length in rats and rams and boars is approximately 3.4, 50 and 50-100m (Franca *et al.,* 2008). The convoluted duct of the epididymis of very long (bull: 36m; boar: 54m) (Hafez, 2000). Development of the epididymis is under the control of testosterone prenatally and its growth increases around the time of puberty when testosterone concentrations increase (Walker *et al*., 2004).

2.5.4 Seminiferous tubes

The seminiferous tubes are formed by the sertoli cells, which provide structural support for the germinal and peri-tubular myoid cells, which surround the tubes (Gnessi *et al.,* 1997). In pigs seminiferous tubule diameters ranges from 160 to 350μm (Walker *et al.,* 2004) and the seminiferous epithelium height (67.5μm) found for wild boars and inserted in the interval related for domestic animals, 60 to 100mm (França & Russell, 1998). The seminiferous tubules total length is a parameter dependent on the testis weight and the seminiferous tubules volume (França *et al*, 2008). In the seminiferous tubules, only sertoli cells possess receptors for testosterone and FSH. Thus, these cells are the major targets of the ultimate hormonal signals that regulate spermatogenesis (Walker *et al*., 2004).

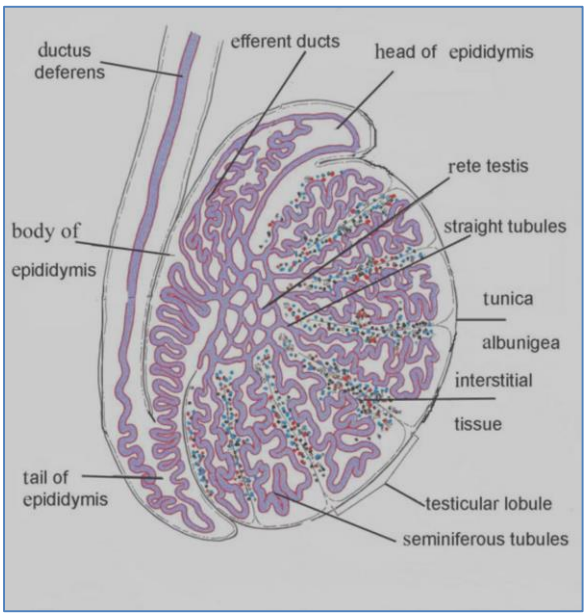

Figure 2.2: General overview of the histological organization of testis and epididymis of male pigs (Gnessi *et al.,* 1997).

2.5.5 Leydig cells

Leydig cell development and function is dependent on pituitary support in late gestation and postnatal (Walker *et al.,* 2004). The stimulatory estrogen feedback mechanism is sexually dimorphic in the miniature pig at 14 days of age (Gnessi *et al.,* 1997). Secretion of testosterone by leydig cells and production of androgen-binding protein by sertoli cells are essential for spermatogenesis maturation of testosterone (Walker *et al*., 2004). Leydig cells are the most important source of androgen (Hansen & Lewis, 1993). LH binds to receptors on the surface of Leydig cells in the testis and stimulates the production of testosterone, a steroid hormone that diffuses into the seminiferous tubules (Walker *et al*., 2004).

2.5.6 Sertoli cell

Sertoli cells are the only somatic elements within the seminiferous tubules (Ugwu *et al*., 2009). The functions sertoli cells have nutritive, protective and supportive for spermatogenic cells (Hansen & Lewis, 1993). Sertoli cells provide a specialized, protected environment within the

seminiferous tubules of the testis for germ cell development (Walker *et al.,* 2004). Sertoli cells provide factors required to fuel germ cell metabolism growth regulatory factors growth factor and hormones that regulate the development of the male reproductive structures or feedback to regulate the hormonal signals affecting sertoli cells (Walker *et al.,* 2005). FSH and testosterone signals that are required to support spermatogenesis are transduced and integrated in sertoli cells (Walker*et al*., 2004. In the male testis, the sertoli cell plays a role in the development and maintenance of spermatogenesis (Ugwu *et al.,* 2009; Umesiobi, 2010). Major function of sertoli cells is to sustain germ cells during development and spermatogenesis (Dyck, 2011).

2.6 Testicular morphology

The reproductive performance of boars is the growth pattern and ultimate size of the testis (Ugwu *et al.,* 2009). Testicular size in boars is relatively genetic (Umesiobi, 2010). Testis size and weight are correlated with daily sperm cell and total sperm reserves (Hansen & Lewis, 1993). The epididymal secretion is the only source of seminal carnitine and concentration in the epididymal plasma of boar ranges from 200 to 300 nmol/mg of protein (McCoard *et al.,* 2003). Weight of testes at a constant age may be a useful indicator trait to select for increased reproductive efficiency of boars (Umesiobi, 2010). Moreover, it has been found to be positively correlated with sperm cell and semen quality in young bulls (Dyck, 2011). Total mass of leydig cells increased during pubertal development, the volume percentage in the testes declined because development of seminiferous tubules was very rapid (Hansen & Lewis, 1993). The important of the parameter evaluating testis function is the determination of the sertoli cell efficiency, which is the best indicator of spermatogenic efficiency (Almeida *et al*., 2006). Sexually mature boars not only synthesize androgens but also high amounts of estrogens. Androgens play a synergistic role to support accessory sex gland function, boar's growth characteristics, and behaviour (Haeussler *et al*., 2007). Furthermore, McCoard *et al*. (2003) reported that it is necessary to develop methodologies that either predict adult testicular size at a young age, such as by marker-assisted selection, or enhance adult testicular size and thus sperm cells.

2.6.1 Factors affecting testicular morphology in pigs

The pig's capacity for protein accretion is the major determinant of growth performance and dietary amino acids and energy are the most influential nutrition controls (Boyd *et al*., 2000). The potential rate of lean tissue gain has improved at a faster rate than growth rate achieved in practice (Boyd *et al*., 2000). The increase in body protein to lipid ratio means that the dietary lysine to energy relationship must be increased or growth rate (ADG) and especially feed conversion efficiency (FCR) will be compromised (Boyd *et al*., 2000). The efficiency of lysine utilisation may decline at increasing body weights. The reduction in trimmed pork weight is consistent with the observed reduction in performance during the finishing period in pigs fed the low protein diets (Tuitoek *et al*., 1997). The effects of growth in body composition have reported a variety of responses ranging from an increase in protein to fat deposition (Hacker *et al*., 1994). The ability of exogenously administered pig growth hormone (pGH) to reduce feed consumption, maintain and increase rate of gain, improve productivity of feed utilization for improvement, reduce the accretion of adipose tissue, and increase accretion of lean tissues has been convincingly demonstrated (Klindt *et al.,* 1998). The boars on the low feeding level lost large amounts of body fat (Kemp & Nicoline, 2001). There is almost no information on what the ideal growth rate and body condition of Kolbroek boars.

2.7 Factors affecting reproductive performance of Kolbroek boars

The effects of libido, quality of sperm, fertility of the sperm cell, welfare, and environmental factors have been reported (Umesiobi, 2008; Wilson *et al*., 2004). The quantity and quality of nutrition with a growth phase can affect the rate of change in the body relationship and the development of different tissue and organs (Coetzee & Casey, 2009). A lower level of nutrition has reduced in semen volume and total sperm cell, while higher nutrient levels increasing semen volume and sperm cell & normal (Whitney *et al*., 2003). The quality of semen is more influenced by other factors related directly or not directly to the level and quality of feeding (Sotirov *et al*., 2002). Nutrition of the boar is important for sexual development and boars mating performance at puberty and it is known that a wide variation in nutrition can be tolerated by mature boars without any negative effect on sperm cell (Hacker, 1994). The feeding method

reduced growth rate and decreased sperm cell of the boars (Sotirov *et al*., 2002) such as *ad libitum* and restricted feed. Nutritional and hormonal factors are major determinants of animal growth, but the mechanisms of how protein influence the hormonal control of protein accretion in growing animals remains relatively undefined (Hansen & Lewis, 1993).

2.8 Protein digestibility

Protein digestibility is a method of evaluating the protein quality based on both the amino acid requirements of humans and their ability to digest it. .Protein digestibility deals with the quantity of absorbed nitrogen or amino acids following protein consumption (Linn, 2005). Though several in vitro methods requiring enzymatic hydrolysis have been proposed, the classical approach uses in vivo digestibility in an animal model or in humans (Linn, 2005). The nutritional value of dietary proteins is related to their ability to satisfy nitrogen and amino acid requirements for tissue growth and maintenance. Ingestion of sufficient dietary energy and protein is a prerequisite for muscle protein synthesis and maintenance of muscle mass and function (Sotirov *et al.,* 2002). Protein digestibility is affected by a number of factors that are related to the feed, the animal, or management (Rivest *et al*., 2000). Protein-bound amino acid absorption may be reduced in complete diets containing low protein concentrations and might contribute to impaired growth performance (Linn, 2005). It is estimated that when growing and finishing pigs are fed low protein diets there is a 30% reduction in nitrogen excretion (Hansen & Lewis, 1993). A nutritional intervention, muscle protein accretion occurs when the rate of muscle protein synthesis is higher than the rate of muscle protein breakdown. A change in the rates of muscle protein synthesis and breakdown can be reflected in the response of muscle protein balance (Rivest *et al.,* 2000). The primary nutritional factors that impose constraints on nitrogen retention of growing pig are protein intake, protein quality, and energy intake (Hansen & Lewis, 1993). Although it is well known that both nutritional and non-nutritional factors influence nitrogen retention, there is little information about how these various factors interact (Hansen & Lewis, 1993) in the South African indigenous Kolbroek pigs.

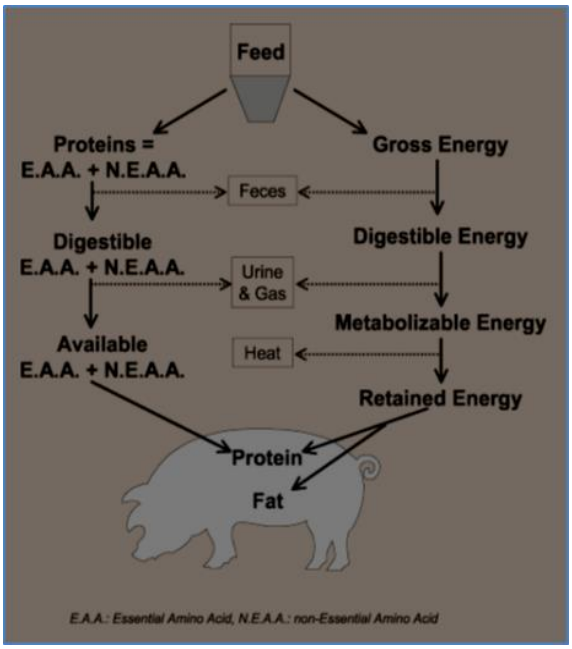

Figure 2.3: Protein digestibility of pigs (Hansen & Lewis, 1993)

2.8.1 Factors affecting digestibility

(a) Rate of passage

The slower the rate of passage, the longer the nitrogenous compounds is exposed to the action of the proteolytic enzymes (Hansen & Lewis, 1993). Processing of feed plays an important role in pig production, especially on small-scale farms. Fermentation of food for human consumption has a long tradition in South-East Asian countries, but this technology has yet limited application in the animal production sector (Rivest *et al.,* 2000). It has been shown that fermented diets may improve growth performance (Scholten *et al*., 1999) and health (Coetzee & Casey, 2009) in pigs in comparison with non-fermented diets. According to Hansen & Lewis, (1993) found an improvement in the in vitro digestibility of organic matter and crude protein due to fermentation.

(b) Crude fibre

To determine the effect of level and source of crude fiber in relation to crude fibre digestibility in pigs, some studies (Sauer *et al*., 1991) have shown that the inclusion of fiber in the diet, to a

certain extent dependent on the level and source, crude protein. The large physiological potential of pigs for fiber utilization has also been demonstrated (Sauer *et al*., 1991). However, modern pig breeds selected for body weight gain and efficient to utilization feed, when fed high-concentrate, low-fiber diets, may have lost intestinal processes required to adapt to high-fiber diets (Rivest *et al.,* 2000). The more the crude fibre in the diet, the more there is dry matter passing to the large intestine to be used as substrate for microbial growth. The greater the dietary crude fibre, the greater the microbial transformations of nitrogen in the hind gut, resulting to a greater error in measuring amino acid digestibility using total faecal collection (Hansen & Lewis, 1993). High fibre diets given *ad libitum* usually cause reduced carcass fat measurements or increased carcass lean, but there have been a few exceptions (Hansen & Lewis, 1993). When dietary crude fibre exceeds 10-15% of the diet, feed intake may be depressed because of excessive bulk or reduced palatability (Adesehinwa, 2008). However, diets or ingredients with high fiber content may negatively affect voluntary feed intake and nutrient digestibility in pigs. The effects of total daily feed level in the diet on nutrient digestibility and feed intake are critical for optimal pig production (Zhang *et al.,* 2013).

2.9 Carcass characteristics

2.9.1 Lean meat percentage

Lean meat percentage (LMP) exemplified by low back fat thickness is an important carcass quality characteristic because it is the criteria for carcass classification (Umesiobi, 2009). The average lean meat percentage ranges from 55 to 60%. For lean meat measurement most countries have used probes based on indirect measurements of back fat as well as loin eye area depth (Tiwari *et al.,* 2013). The pork producer must be concerned about production of edible pork which is acceptable by the consumer. Pork quality is very important not only to consumers but also to food industry (Tiwari *et al*., 2013). Today's consumers are more health conscious than in previous decades and this leads to a preference for lean meat (Klont *et al*., 1998). The rate of protein deposition decreases and more of the consumed energy, over and above maintenance energy requirements, is directed toward body lipid deposition (Klont *et al*., 1998). The rate of body lipid deposition is correlated with the fatty acid composition of adipose

tissue. Back fat thickness decreases the level of unsaturated fat content of the adipose tissue increases (Walker *et al*. 2004).

2.9.2 Dressing percentage

Dressing percentage is an important factor in determining production efficiency. Dressing percentage is calculated by dividing the chilled carcass weight by the live weight and multiplying by 100 (Tiwari *et al.,* 2013). The optimum weight is a matter of growing pigs to the correct weight determined by the marketing grid. Markets in pigs are sorted by live weight, most grid are measured in carcass weight, it is important to accurately estimate dressing percentage. Dressing percentage, carcass yield, is determined by dividing the carcass weight by live weight (Nissen & Oksbjerg, 2011). The importance of pig is highest feed conversion efficiency, can utilize wide range of feedstuffs, highly prolific with shorter generation interval, requires small investment on building and equipment and dressing percentage ranges 65-80% in comparison to other livestock species (Tiwari *et al.,* 2013).

2.9.3 Carcass Quality

A large individual variation exists in meat quality, both within and between animals of the same breed, sex and environment (Klont *et al*., 1998). There are also some differences between fibre composition of different muscles and between animals, which may influence meat quality and depend on factors such as body, age, weight, and breed (Klont *et al*., 1998). An important part of meat quality in relation to consumer acceptance is tenderness and (Nissen & Oksbjerg, 2011) indicated that the low protein diet can have an impact on protein turnover in the muscle and consequently affect meat tenderness. Meat quality trait is often thought to be unique and independent, in reality most of the commonly measured meat quality traits are associated, or correlated, with each other. In the pig industry they focused on improving pork quality (Matthews *et al*., 2001). Muscle fibre characteristics have a profound influence on meat quality and carcass quality (Dai *et al.,* 2009).

2.9.4 Carcass length

The carcass length is an important factor that determines the number of back bacon rashers that may be obtained from pigs. The carcass length of exotic breeds is usually higher than those of indigenous breeds. The carcass length is measured from the first rib to the pelvic bone using a measuring tape (Schinckel *et al.,* 1996). The back fat content of pigs is measured to determine the amount of fat in the pork (Csato *et al*., 2002). Back fat measurements are done with an ultrasound in live animals and vernier callipers are used to measure back-fat in carcasses (Hoffiman *et al*., 2003). Back fat thickness measured off the midline to be more accurate indicator of carcass muscling and fatness measurements are taken on live animals using ultrasound scanning (Csató *et al*., 2002).

2.10 Protein accretion

Protein accretion (PA) is the balance between protein synthesis and protein degradation (Umesiobi, 2009). Protein accretion in pigs muscle is dependent on both muscle protein synthesis and protein degradation such that protein accretion is equal to protein synthesis minus protein degradation (Bergen *et al.,* 2008). Protein accretion is determined by the pig's nutrients requirements for growth, composition of growth, and response for nutrition or management change (Hansen & Lewis, 1993). In commercial herds and more economical method are easier to predict protein accretion rates to estimate fat-free lean gain from 20 kg to slaughter. Protein accretion rates (PAR) were predicted as the product of the derivatives of live weight growth and protein mass to live weight (Schinckel *et al.,* 1996). In growing pigs, is important consuming energy to maintain is the requirement for protein accretion (Boyd *et al*., 2000). The capacity for protein accretion is determined by genotype, gender, and stage of growth and forced by inadequate energy intake (Boyd *et al*., 2000). The impact of protein accretion increase in the daily maintenance energy requirement for the growth period depends on the time to reach the growth end-weight (Boyd *et al*., 2000). Growth and protein accretion of an animal depends on the rate of protein synthesis exceeding that of protein breakdown (Bergen, 2008). Nevertheless, whether muscle protein synthesis is decreased in the post

absorptive state, a significant part of daily life is spent in the fed state, and feeding is an important regulator of muscle protein synthesis (Campell *et al.,* 1990).

Testosterone administration increases protein accretion in rat muscle by increasing protein synthesis. Pigs with accelerated growth rates may have delayed maturity and that protein synthesis rate changes with age, thereby necessitating carrying out measurements at various points on the growth curve (Hansen & Lewis, 1993). Pig hormones can act directly on adipose tissue to inhibit lipo-genesis and the resulting increase in energy available for growth may play a key role in stimulating protein accretion (Campell *et al*., 1990). The anabolic effects of exogenous pGH in pigs are unequivocal, but there is no published information on the changes in protein synthesis and breakdown that lead to this response (Hansen & Lewis, 1993). Therefore, is needed to evaluate the effect of protein accretion on the reproduction performance of Kolbroek bars.

2.11 CONCLUSION

When more protein synthesis are absorbed by pigs, it leads to building of the tissues muscles. It is thus, imperative to measure backfat thickness on the live pig to evaluate how much fat is being composed. The average age of puberty of boar is approximately 5-8 and 7 months. Reduced energy and protein levels in boars delay puberty and sexual development, sperm cell and libido.

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Chapter 3

EFFECTS OF DIETARY PROTEIN LEVELS ON GROWTH PERFORMANCE AND BACK FAT THICKNESS IN KOLBROEK BOARS

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3.1 ABSTRACT

There is no standardised level of protein requirements for Kolbroek boars. The objective of the study was to evaluate the growth performance and back fat thickness in Kolbroek boars fed diets containing different levels of protein. A total of 14 Kolbroek boars were recruited from birth and used for this study. Day old (1) boars were randomly allocated to three protein diets, Diet 1 (n=5); 2 (n=4) and 3 (n=5) comprising of 10, 13 and 16% protein. Kolbroek pigs were on creep feeding diet as ad libitum from Day 10 until weaning (Day 42). After weaning boars were given creep feeding for four weeks. Weighing indicator was used to determine the body weight of Kolbroek boar on weekly basis. There was no significant effect of the dietary protein on growth performance of Kolbroek boars. However, there was a positive correlation between feed intake and average daily gain (r= 0.78). Moreover, a positive correlation existed between average daily gain and back fat thickness (r= 0.12). However, there was a negative correlation between feed conversion ratio and average daily gain (r= -

0.94). Interestingly, there was a negative correlation between feed intake and feed conversion ratio (r= -0.57), feed intake and back fat thickness (r= -0.08), FCR and back fat thickness (r= -0.02). In conclusion, there was no significant difference on 10, 13 and 16% of protein on growth performance of Kolbroek boars. However, it is suggested that 10% protein used for Kolbroek boars. Furthermore, a strong correlation existed between average daily gain and feed intake. It is thus recommended that more studies should be conducted on how to reduce fat content of the breed.

***Keywords**:* Performance traits, protein diet, indigenous boars

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3.2 INTRODUCTION

The origin of South African indigenous Kolbroek pig display some similarities to the typical Chinese pig breeds and is well-adapted to harsh conditions, easy to handle and do not show any outstanding susceptibility to stress (Nicolas, 1999). Moreover, there is speculation that the name of the so-called Kolbroek pig breed is derived from the Coalbrook, a ship of the British East India Company which was wrecked at Cape Hangklip, close to Betty's Bay, in 1778. The ship had pigs on board which were rescued and it is surmised that the name Kolbroek originated from these animals. These pigs are characterised by their "spotted" colour (Hoffman *et al.,* 2005). Kolbroek pigs are small sized, weighing about 0.5 kg at birth and about 45.5 kg at one year of age (Anugwa & Okwori, 2008). They have been recommended as a good alternative source of cheap, high quality animal protein that suits the accelerated human population (Ironkwe & Amefule, 2008). However, Kolbroek pigs are often seen as less efficient because of their tendency to put on excess fat (Ramsay *et al*., 1994). Consequently, these may be due to the fact that there is no standardised protein requirement for Kolbroek boars. To date, information concerning the effect of protein levels on growth performance of Kolbroek boars is not yet studied. Thus, there is a need to evaluate the effect of protein on growth performance of Kolbroek boars.

Body weight of a boar is an important indicator of its growth, health and readiness to go to market (Wang *et al*., 2008). Low nutrient levels can easily be met through use of locally available feed resources, such as leguminous leaf meals, groundnut hulls, sunflower cakes and other fibrous protein sources (Chimonyo *et al*., 2005). Nutrient status influences libido, structural soundness and longevity, sperm production, and semen quality. Many farms feed boars on sow diets, but this feeding program may neglect some of the unique nutrient requirements that must be accounted to optimize breeding herd performance in working boars (Whitney *et al*., 2004). Consequently, a low protein diet has been shown to further reduce boar interest in producing ejaculate (Louis *et al*., 1994). Thus, feeding a proper nutritional program will result in improved reproduction performance of the boars.

Large White and Landrace pigs fed 16% protein levels decline in growth rates (Anugwa & Okwori, 2008). In contrast, boars fed 9% protein diet reduced amino acid deficient compared to pigs fed the 18% CP diet, which showed increased in growth rates (Louis *et al*., 1994). Back fat thickness is one of the most important economic traits that are used to assess the quality of pork. Back fat thickness of exotic pigs have been studied intensively but little information is available on back fat thickness in indigenous pigs and their Large white Yorkshire crosses in India (Lakhani & Jogi, 2000). Hence, the objective of the study was to evaluate the growth performance, age of attainment of puberty and weight at puberty of Kolbroek pigs fed different levels of protein diet.

3.3 MATERIALS AND METHODS

3.3.1 Experimental site

The study was conducted at the Pig Research Unit of Agricultural Research Council Animal Production Institute, Irene, South Africa. The Agricultural Research Council-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 m above sea level. Before the commencement of this study, the project experimental protocol was evaluated and approved by the Animal Ethics

Committee of the Agricultural Research Council-Animal Production Institution, with reference number APIEC13/002.

3.3.2 Experimental animals

A total of 14 Kolbroek boars of 12 weeks old (±13.4kg) were selected and used in this trial. Diets containing 10%, 13% and 16% CP were formulated. Pigs were weighed on an internal using scale. Pigs were kept until 12 weeks of age (live weight. The diets comprised of 10% (n=5), 13% (n=4) and 16% (n=5) protein diets. Kolbroek pigs were fed creep feeding diet as ad *libitum* from Day 10 until weaning (Day 42). After weaning boars were given punch creep feeding for four weeks. Weighing indicator was used to determine the body weight of Kolbroek boar on weekly basis.

3.3.3 Experimental diets

Kolbroek boars were weighed (weighing scale indicator) as from the first week of birth until the end of the experiment. The room temperature was determined by using thermometer ranging from 22-25^{0}C. Each of the boar treatment groups were kept in a pen and each pen was equipped with a self-feeder and an automatic water nipple (Webb *et al*., 2006). Diets were formulated to meet ARC requirements by using Format international- feed formulation software solutions (FI-FFSS). Water was provided *ad libitum*. The experimentally formulated diets are presented in Table 3.1.

Table 3.1 Chemical composition of three protein diets

Ingredient (g/Kg)	10% protein diet	13% protein diet	16% protein diet
Wheat bran	151.3	155.4	159.4
Hominy chop	300.0	325.2	350.8
Maize meal	495.7	399.0	304.1
Soya oil cake	00.0	0.0	121.4
Monocalcium	25.0	16.5	7.9
FFS Macro	10.0	20.0	30.0
Limestone	6.0	9.5	13.0
Salt	1.0	1.0	1.0
Lysine HCL	6.0	6.9	7.8
DL Methionine	1.0	1.0	1.0
Pig Supplement	4.0	4.0	4.0

FFS- full fat soya, HCL- Lysine Hydrochloride

3.3.4 Experimental measurements

Average daily feed intake (ADFI) was measured daily. Body weights (BW) were recorded on a weekly basis prior to feeding to estimate average daily gain (ADG). Feed conversion ratio (FCR) was calculated as the ratio of feed intake of the experiment unit was divided by the total weight gain per pen. Feed intake was recorded individually. The amount of feed supplied to pigs was weighed and recorded. To calculate feed intake, the amount of feed inside the feeding trough after every seven days was subtracted from the total feed supplied for the seven days. The average daily feed intake was calculated by adding feed intakes for the entire experimental period and then dividing by the total feed intake by the number of experimental unit was divided by the total weight gain per pen. All Kolbroek boars were weighed weekly to determine their ADG. Backfat thickness of the live pigs were measured weekly by means of a Renco ultrasound

P2 backfat probe supplied by Instavet SA (Webb *et al*., 2006). Kolbroek boars were fed ad *libitum*, the feed intake and residual feed were weighed every day.

3.4 DATA ANALYSIS

Data were analysed using one way analysis of variance (ANOVA). Shapiro-Wilk's test was performed to test for non-normality (Shapiro, 1965). Student's t-Least Significant Difference (LSD) was calculated at the 5% level to compare treatment means of significant effects.

3.5 RESULTS

The effects of protein levels on growth performance of pigs are summarized in **Table 3.2**. No significant difference was observed on growth performance, irrespective of the protein diet. Kolbroek boars fed diet containing 13% crude protein exhibited body weight that were numerically higher (84.6 kg) than those experimental group that were fed diet with 16% of crude protein with (82.3 kg) and 10% protein diets (78.3 kg). Kolbroek boar fed 16% of protein diet consumed more (1.5 kg) and gain less (69.9 kg) than those pigs fed 13% of protein with (1.4 kg), but statically there was no significant different. The pigs fed the 13% protein diet also gained more weight (71.2 kg). The back fat thickness increased rapidly with increasing body weight in all experimental groups, while there was no statistical significant different between experimental groups on the initial and final weight. The final weight and ADG of the pigs were not different among dietary treatments. There was no significance different on age at puberty and weight at puberty among the treatment. It is of advantage for small scale farmer because 10% protein diet might be affordable than other diet which is high levels.

Table 3.2 Effects of protein diet levels (mean (±SD) on growth performance of Kolbroek boars

Parameter (Kg)	Levels of protein		
	10% protein diet (n=5)	**13% protein diet (n=4)**	**16% protein diet (n=5)**
Initial weight kg	12.1 ± 3.4	13.4 ± 2.6	12.4 ± 3.6
Final weight kg	78.3 ± 11.8	84.6 ± 4.4	82.3 ± 3.4
Total gain (kg)	66.2 ± 10.8	71.2 ± 2.1	69.9 ± 2.7
ADG (kg/day)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
ADFI intake (kg/day)	1.4 ± 0.1	1.4 ± 0.0	1.5 ± 0.0
FCR	4.4 ± 0.5	4.2 ± 0.1	4.4 ± 0.2
Back fat thickness (cm)	18.2 ± 0.8	18.8 ± 1.3	18.0 ± 1.2
Age at puberty (day)	157.2±5.7	153.8±4.3	154.4±4.1
Weight at puberty(kg)	41.2±8.4	44.1±4.1	43.8±4.1

ADG- average daily gain; ADFI- average daily feed intake; FCR-feed conversion ratio

Pearson correlation coefficients between growth performances of Kolbroek boars are summarized in **Table 3.3**. There was a high positive correlation between ADFI and ADG (r= 0.78). Moreover, a positive correlation existed between ADG and back fat thickness (r= 0.12). In contrast, there was a negative correlation between feed conversion ratio and average daily gain (r= -0.94). Furthermore, negative correlations existed between feed intake and FCR (r= -0.57), feed intake and back fat thickness (r= -0.08); FCR and back fat thickness (r= -0.02).

Table 3.3 Pearson correlation coefficients in growth performances of Kolbroek boars

Parameters	ADG (g/day)	ADFI (kg/day)	FCR	Back fat thickness (cm)
ADG (kg/day)	1.00			
ADFI (kg/day)	0.78	1.00		
FCR	-0.94	-0.57	1.00	
Back fat thickness (cm)	0.12	-0.08	-0.02	1.00

ADG- average daily gain; ADFI- average daily feed intake; FCR- feed conversion ratio

3.6 DISCUSSION

This study demonstrated that there was no significant difference between 10, 13 and 16% protein diets on growth performance of Kolbroek boars. When Kolbroek boars were fed 13% protein, their body weight was numerically higher (84.6 kg) than 10% (78.3) and 16% (82.3kg) diet group. There was a positive correlation between feed intake and ADG (r= 0.78). Moreover, a positive correlation existed between ADG and back fat thickness (r= 0.12). In contrast, there was a negative correlation between FCR and ADG (r= -0.94).

The study showed that there was no significant effect on growth performance of Kolbroek boars, irrespective of the protein diet. Similarly, Iheukwumere *et al.,* (2008) indicated that the growth performance of Large White and Landrace boars fed diets containing different protein was not affected. Feed intake was not affected by diets in the Kolbroek boars. Correspondingly, it was indicated that feed intake was not significantly affected by different protein diets (Anugwa & Okwori, 2008; Kerr *et al.,* 1995; Kephart & Sherritt, 1990). In the current study, there was no significant effect on the bodyweight of Kolbroek boars, irrespective of the protein diet fed. This is in agreement with previous studies whereby no significant difference was observed for boars fed 13 and 16% protein diets (Sirtori *et al*., 2010; Iheukwumere *et al.,* 2008).

There was no significance different between protein levels on FCR, ADG and ADFI in Kolbroek boars. Similarly, FCR was not significantly affected by 12 and 16% diets (Anugwa & Okwori, 2008). Furthermore, no differences were observed for ADG, ADFI and feed efficiency when 10, 12 and 14% protein diets were fed to boars, irrespective of the breed (Tuitoek *et al*., 1997; Wu *et al.,* 2010; Lee *et al*. 2000). Back fat thickness increased rapidly with increasing body weight in pigs fed 10, 13 and 16% of protein. Kephart & Sherritt (1990) reported that protein levels of 14, 16 and 18% diets do not have an effect on back fat thickness.

No significant difference was observed on age of attainment of puberty, weight at puberty, irrespective of the protein diet. Similarly, Kuehn *et al.* (2009) reported that there was no significant difference between the times for first erection and first mount at puberty. Similarly, Schulz *et al.* (2004) reported that there were no differences in the number of mounts, intromissions, or ejaculations were observed between two groups of males. Similarly, Ewuola & Egbunike (2010) reported that the rate at which the animals attained puberty was not significantly different among the treatments. Similarly, Labroue *et al.,* (1997) reported that body weight was not affected by levels of protein diets.

There was a high positive correlation between ADFI and ADG (r= 0.78). Similarly, it has been reported that there is a strong correlation between ADFI and ADG (Hoque & Suzuki, 2009; Hyun & Ellis, 2002; Labroue *et al.,* 1997). There was a negative correlation between FCR and ADG (r= -0.94) as well as FCR and ADFI (r= -0.57). In contrast, it was reported there was a relatively low correlation between FCR and ADG (r= 0.27) as well as FCR and ADFI (r= 0.31).

37 CONCLUSION

Diets containing different CP levels did nontly produce any significant effects on growth performance of Kolbroek boars. However, there was a strong correlation between average daily gain and feed intake. Furthermore, a negative correlation exists between ADG and FCR as well as FCR and ADFI. Further studies are required to study fat content of Kolbroek boars.

3.8 RECOMMENDATION

The 10% of protein diet is recommended for Kolbroek boars, Kolbroek boars require less protein diet (10%) as body weight, feed intake, FCR, backfat thickness are also the same with 13 and 16% of protein.

3.9 ACKNOWLEDGEMENTS

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Chapter 4

THE EFFECT OF DIFFERENT DIETARY PROTEIN CONCENTRATION ON ATTAINMENT OF PUBERTY AND LIBIDO IN KOLBROEK BOARS

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4.1 ABSTRACT

The objectives of the study were to determine the effect of different dietary protein levels on libido status, gonadal measurements, and age of attainment puberty in Kolbroek boars. A total of 14 Kolbroek boars at day old (1) were used as described in the previous chapter. Kolbroek boars were randomly allocated into three protein diets comprising of 10, 13 and 16%. Body weight, backfat thickness, libido status, hormonal concentrations and reaction times were measured from 5-8 months of age. The sexual behavior traits evaluated were time anogenital sniffing (TAGS), time nosing side (TNS), elapsed time to first mount (ETFM), time side and rear mounted with penis not exposed (TMNP), time side and rear mounted with penis exposed (TMWP) and elapsed time to copulation (ETC). These behavioral characteristics were recorded separately to the nearest second for the duration of evaluation Gonadal measurements increased with age, irrespective of diet. Body weight (BW), back fat thickness, gonadal

measurements and hormonal concentrations were not affected by diets. However, boars fed 13% diet had more reaction time compared to boars fed other diets. Protein inclusion levels had no significant effect on the body weight, testosterone levels and gonadal measurements.
Key words: Kolbroek boars, plasma concentration, testosterone, libido, puberty and protein.

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4.2 INTRODUCTION

Puberty is the time at which a maturing male starts to exhibit mounting behavior and penal erection, able to produce sufficient spermatozoa (Brown, 1994). It has been documented that Kolbroek pigs reach puberty at early stages of 5 months and grows slower than modern pig breeds (Chimonyo *et al*., 2005). Indigenous boars reach puberty early, have smaller adult testes and produce less sperm than commercial crossbred boars such as Large White and Landrace (Ford & Wise, 2009). Furthermore, it has been reported that indigenous pigs, breeds such as Mukota, reach puberty at about 4 to 5 months of age (Trudeau *et al*., 1992; Chimonyo *et al*., 2005). However, there is little information about the age of attainment puberty, libido status and gonadal measurements.

The onset of puberty is highly variable between individual boars and is influenced by factors such as nutritional status (Brown, 1994), stress, social rank (de Jonge *et al*., 1996) and breed (Babol *et al.,* 2004; Olugbenga & Samuel, 2007). According to Coetzee & Casey, (2009) puberty is reached within a close range of body fatness even if growth had been depressed by nutritional factors. Furthermore, the efficiency of dietary protein intake for growth increases and pubertal growth rate is achieved by an increase in the efficiency of protein utilization (Beckett *et al.,* 1997). However, it has been reported that selecting for high lean growth in pigs tends to delay puberty in pigs (Hills *et al*., 1990 & Whittemore, 1998) as opposed to indigenous pigs breeds.

Libido evaluated by reaction time (Umesiobi & Iloeje, 1999) is expressed from puberty and maturation and it persists at a fairly constant level for the remainder of the animal's lifespan

(Boyd *et al*., 1996; Umesiobi, 2010). Libido is primarily dependent on the production of testosterone by the testis (Turkstra, 2005). Boars fed at high rate are expected to have increased libido problem (Dritz *et al.,* 2006). In contrast, it has found that higher feed intake increases sperm production and libido (Boyd *et al.,* 1996). Moreover, Louis *et al.* (1994) indicated that boars fed the low protein diet take longer to show sexual behavior.

Testosterone is an androgen produced in the testes that stimulates protein synthesis and reduces protein degradation (Ford & Wise, 2009). Testosterone concentrations decrease and remain low during further fetal development (Haeussler *et al.,* 2007). However, assessment of scrotal circumference in pubertal boars is not practiced routinely, due to their pendulous scrotum with its broader attachment (Ford & Wise, 2009). In male mammals, Luteinizing Hormone the synthesis and secretion of androgens from the leydig cells in the testes. A Follicle Stimulating Hormones is responsible for the initiation of spermatogenesis and its play a role with testosterone inhibits LH and FSH secretion from the pituitary (Turkstra, 2005). The importance of this increase in steroid oogenesis on steroid production at puberty is unknown (Ford & Wise, 2009). Therefore, there is a need to evaluate the effect of protein diets on the hormonal concentration of Kolbroek boars. The objectives of the study were to evaluate the age of attainment of puberty, weight at puberty and libido test of kolbroek pigs fed diets containing different levels of proteins.

4.3 MATERIALS AND METHODS

4.3.1. Experimental site

The study was conducted at the Pig Research Unit of Agricultural Research Council, Animal Production Institute, 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 region of RSA and situated at an altitude of 1525m above sea levels. The protocol of the experiment was evaluated and approved by the Animal Ethics Committee of the ARC-API before the experiment could be initiated (APIEC13/002).

4.3.2 Experimental animals

Fourteen (14) Kolbroek boars at the age of 5 months were randomly allocated to three experimental diets as detailed in previous. Kolbroek boars were fed individually 1.5 kg of diets per day. The attainment of puberty and weight at puberty of boars were evaluated and recorded. Trail. The previous experiment showed that the final BW of pigs ranges from 78-84 kg. After the initial period of training of 4 weeks in determining the age of puberty, the boars were mounting a dummy sow. During this period up to 8 months old, the boars were monitored twice a week for attainment of puberty. Boars were allowed access to a dummy sow and were considered to have reached puberty when they mounted the dummy sow. Four blood samples were taken at 15-min via ear vein. All training for sexual behaviour was done by Mondays and Fridays in the morning and was performed by the same trained technician.

4.3.3 Experimental measurements and observation of sexual characteristics

Right and left testes of the Kolbroek boars were measured and recorded using Vanier calliper in millimetre (mm). Boars were kept at room temperatures (24°C) and temperatures was recorded daily by using a thermometer (22-25^{O}C). Sexual behaviour was assessed twice per week until the age of 8 months: reaction time (the interval between entering the collection pen and first interaction with artificial sow), the interval between entering the collection pen and the start of ejaculation, duration of ejaculation and number of false mounts (mounting artificial sow but dismounting before allowing a complete collection of semen). The sexual behaviour characteristics evaluated were: time anogenital sniffing (TAGS), time nosing side (TNS), elapsed time to first mount (ETFM), time side and rear mounted with penis not exposed (TMNP), time side and rear mounted with penis exposed (TMWP) and elapsed time to copulation (ETC). These behavioural characteristics were recorded separately to the nearest second of time for the duration of evaluation as described by (Levis *et al.,* 1997).

4.3.4 Blood collection

Subsequently, Kolbroek boars were bleeding once per week at 09h00 to 11h30 for 15 minutes individually. Blood samples were collected in 10 ml tubes containing heparin tubes. The 19-gauge needles were inserted into the ear vein and blood was collected into 10 ml syringes. All blood samples were centrifuged at 1 500 x g for 10 min and serum decanted and stored at -20°C until analysis for testosterone, FSH and LH. All samples were analysed for plasma hormonal concentrations using a commercially available RIA kit (Diagnostic Systems Laboratories, Webster, TX) validated for porcine serum.

4.4 DATA ANALYSIS

Data were analysed using SAS, (1999): Student's t-Least Significant Difference (LSD) was calculated at the 5% level to compare treatment means of significant effects.

4.5 RESULTS

The results for the effect of protein diets on sexual behaviour (mean age, body weight at puberty, and testes (length and width) are presented in Table 4.1. The rate at which boars attained puberty was not affected by dietary treatments. The effect of dietary crude protein levels on hormonal secretion of Kolbroek boars is presented in Table 4.3**.** There was no significant difference on the testosterone levels in boars during the 5, 6 and 8 months of age. However, the boars on the diet contains 10% CP had higher levels of testosterone compared to those fed the other diets. Progesterone, FSH and LH levels were not affected by Dietary treatments.

The effect of dietary crude protein levels on testicular traits, body weight and backfat thickness of Kolbroek boars are presented in Table 4.4. There were no difference on length on both the left and right testicles at 5, 6 and 7 months of age, however at 8 months. There were no significant different between protein levels on left testicles length, left width, right width of 5, 6, 7 and 8 months. The effect of dietary protein levels on hormonal of Kolbroek boars presented in Table 4.3. There was no significant different between protein levels on testes, progesterone, FSH, LH in Kolbroek boars. Table 4.2. The effects of dietary crude protein levels on sexual

behaviour of Kolbroek boars at puberty until 8 months of age (±LSD) and seconds. At 7 and 8 months of age there was no significant different in different protein levels. There was a significant different between 10, 13 & 16 % protein diet and on the TMWP there was a significant between 10, 13 & 16% protein diet. TNS (sec), TAGS (sec), ETFM (sec), TMNP (sec), NM, there was a significant different between protein levels on TMWP (sec). This results shows that the duration to mount with penis exposed was increased from 5 to 8 months of age. The duration to mount without penis exposed at 5 months was increased, when the reach the age of 8 months was decreased in all the diets.

Table 4.1 Effects of dietary crude protein levels on reproductive performance in Kolbroek pigs (SEM/SD)

Protein levels	No. animals	Age at puberty (d)	Weight at puberty (kg)
10 %	5	157.2±5.7	41.2±8.4
13 %	4	153.8±4.3	44.1±4.1
16 %	5	154.4±4.1	43.8±4.1

Table 4.2 The effects of dietary crude protein levels on sexual behaviour of Kolbroek boars at puberty until 8 months of age (±LSD) second

Parameter	Protein levels	5 months	6 months	7months	8months
TNS (sec)	10%	87.0± 18.2[a]	138.6±31.2[a]	186.8±16.6[a]	33.4±16.4[a]
	13%	82.0±22.9[a]	138.3±15.2[a]	210.3±13.9[a]	38.0±15.3[a]
	16%	82.8±10.5[a]	163.0±20.3[a]	213.8±28.8[a]	30.0±9.9[a]
TAGS (sec)	10%	93.0±20.5[a]	137.4±20.0[a]	194.8±14.2[a]	34.6±22.2[a]
	13%	98.0±22.3[a]	162.3±22.2[a]	215.8±25.6[ab]	21.8 ±16.8[a]
	16%	98.6±20.2[a]	149.6±23.2[a]	187.4±18.3[b]	30.0±21.7[a]
ETFM (sec)	10%	91.0±13.9[a]	165.6±20.4[a]	200.2±27.1[a]	27.8±14.7[a]
	13%	85.3±13.6[a]	132.0±12.5[ab]	191.5 ±11.7[a]	27.5±20.1[a]
	16%	81.2±14.1[a]	149.0±19.0[b]	200.8±17.0[a]	19.2±11.4[a]
TMWP(sec)	10%	32.6±23.1[a]	122.2±13.6[a]	199.6±33.0[a]	342.2±51.6[a]
	13%	40.0±19.1[a]	123.5±134[a]	187.0±33.5[a]	260.0±58.5[a]
	16%	36.4±10.9[a]	128.4±30.9[a]	191.4±21.0[a]	296.4±65.5[a]
TMNP (sec)	10%	181.8±26.3[a]	156.8±16.5[a]	71.6 ±10.7[a]	44.0±12.7[a]
	13%	187.3±33.1[a]	133.8±23.9[a]	63.5±14.8[ab]	15.3±3.7[b]
	16%	207.8±32.5[a]	140.2±21.9[a]	93.6±23.6[b]	22.4±19.7[b]
NM	10%	1.4±0.15[a]	2.4±1.3[a]	1.2±0.4[a]	2.0±0.7[a]
	13%	2.0±0.8[a]	1.8±1.0[a]	1.8±1.0[a]	1.3±0.0.5[a]
	16%	2.0±0.7[a]	1.4±0.5[a]	1.8±0.8[a]	1.4±0.5[a]

(P > 0.05), TNS-time nosing of dummy sow, TAGS-time anongenital sniffing, ETFM- elapsed time to fist mount, TMNP- time mounts with penis exposed, TMWP-time mount without penis exposed, NM- no. of mounts.

Table 4.3 The effect of dietary crude protein levels on the hormonal of Kolbroek boars (± SD).

Parameter	Protein levels	5 months	6 months	7 months	8 months
Testosterone (ng/ml)	10%	2.9±1.4[a]	4.3±1.6[a]	3.6±1.2[a]	6.3±5.1[a]
	13%	3.6±2.0[a]	3.1±2.0[a]	4.3±1.4[b]	7.2±8.4[a]
	16%	2.6±1.6[a]	5.2±2.1[a]	6.5±2.6[c]	10.4±2.7[a]
	10%	2.6±18.5[a]	14.2±16.8[a]	30.8±38.0[a]	18.0±16.2[a]
Progesterone	13%	42.9±17.3[b]	9.6±8.4[a]	22.0±23.9[a]	27.0±44.8[a]
(ng/ml)	16%	37.7±65.3[b]	24.4±39.7[a]	15.8±22.1[a]	2.0±2.4[a]
	10%	2.0±0.8[a]	3.2±0.5[b]	3.1±0.6[a]	2.8±0.7[a]
FSH	13%	11.6±0.6[a]	2.3±0.6[a]	3.0±0.3[a]	3.1±0.8[a]
(ng/ml)	16%	2.1±0.5[a]	3.5±0.5[a]	0.3±0.2[a]	2.0±1.0[a]
	10%	0.6±0.2[a]	0.2±0.1[a]	0.3±0.2[a]	0.5±0.2[a]
LH	13%	0.6±0.2[a]	0.1±0.1[a]	0.3±0.2[a]	0.6±0.4[a]
(ng\ml)	16%	0.6±0.3[a]	0.2±0.0[a]	0.4±0.1[a]	0.8±0.2[a]

[a-b] Values with different letters of superscript differs significantly (P < 0.05) FSH - Follicle-stimulating hormone , LH - Luteinizing hormone

Table 4.4 The effect of dietary crude protein levels on testicular traits and with body weight and backfat thickness of Kolbroek boars (±LSD).

Parameter	Protein levels %	5 months	6 months	7 months	8 months
Body weight (Kg)	10%	36.4±8.0	45.5±6.3	59.6±7.3	71.4±9.2
	13%	39.5±4.5	48.5±3.8	63.2±4.0	75.2±3.4
	16%	39.6±4.7	48.4±3.5	62.2±3.6	74.5±3.6
Back fat thickness (P_2)	10%	11.6±0.9	14.8±0.4	15.2±1.1	19.0±0.7
mm	13%	11.3±1.0	13.8±1.7	15.0±1.8	19.5±0.6
	16%	11.6±0.5	13.8±0.8	14.2±2.8	19.6±0.5
Right testicles length	10%	8.7±1.4	10.6±1.1	11.2±1.2	11.2±1.1
(mm)	13%	8.1±0.8	11.2±1.1	11.4±1.1	11.6±1.2
	16%	8.6±1.0	9.8±0.2	10.0±0.2	10.2±0.2
Left testicles length	10%	10.0±1.3	12.3±1.1	12.5±1.1	12.9±1.2
(mm)	13%	9.4±0.8	13.8±1.5	14.2±1.6	14.3±1.6
	16%	10.3±1.0	9.3±5.8	12.6±0.2	12.7±0.3
Right testicle width	10%	4.7±0.8	5.0±2.0	6.2±4.5	5.7±0.4
(mm)	13%	4.4±0.5	5.6±0.5	5.7±0.4	6.0±0.2
	16%	4.2±0.4	5.5±0.2	5.6±0.2	5.9±0.3
Left testicle width (mm)	10%	5.0±0.8	5.6±2.0	5.8±0.6	5.8±0.6
	13%	4.8±0.5	5.8±0.4	6.0±0.4	6.0±0.4
	16%	4.3±0.5	5.8±0.1	9.4±7.1	6.7±4.2

4.6 DISCUSSIONS

Dietary treatment did not effect on the age of attainment of puberty, body weight, back fat thickness, hormonal concentration and sexual behaviour in Kolbroek boars. This agree with

Kuehn *et al.* (2009) who reported differences between the time for first erection and first mount at puberty in Large White pigs. Moreover, Schulz *et al.* (2004) reported no differences in the number of mounts, intromissions, or ejaculations between two groups of male boars. Borg *et al.* (1991) reported no significant effects of serum GH and testosterone concentrations on Large White boars that displayed either a high or low mounting frequency.

Levis *et al*. (1997) reported that no significant difference between the elapsed time from first mount of commercial boars. Boars fed the low protein diet required 94% more time to mount the collection dummy and start ejaculating (Louis *et al*., 1994). Louis *et al*. (1994) reported that protein levels did not affect libido status of the Large White pigs.

Similarly, Ewuola & Egbunike (2010) reported that the rate at which the animals attained puberty was not significantly different among the diets. The body weight of Kolbroek boars was not affected by the dietary crude protein content. Also, Levis *et al.* (1997) reported that body weight was not affected by levels of protein diets.

The results showed testosterone only differed at 7 month of age in Kolbroek pigs. In contrast, Louis *et al.* (1994) reported that testosterone and LH concentrations were not affected by protein levels in Large White pigs. However, Naden *et al.* (1990) reported that testosterone was starts at 4-6 months and increased through 56 weeks of age. The current results indicated that FSH declined at 7 and 8 months of the experiment in 10, 13 and 16% protein diet of Kolbroek boars. Trudeau *et al*. (1992) reported that in Landrace X Large White pigs that the LH/FSH ratio declined progressively until the age of 7 months. There were no significantly different between 10, 13 and 16 % protein diet on LH of Kolbroek boars. Testosterone levels of Kolbroek boars increased with age, irrespectively of different protein levels. The levels of testosterone start to increase at 6 months of age until 8 months age in all treatment. At Day 84, testosterone levels increased rapidly until the end of the trial at 5 months (Trudeau *et al*., 1992).

4.7 CONCLUSION

The present study indicate that dietary crude protein levels did not have any significant effects on the age of attainment of puberty, testosterone, FSH and LH, body weight, back-fat thickness, progesterone and libido test of Kolbroek boars. However, findings from this study indicate that 10% of dietary crude protein inclusion into pig diets was most beneficial for poor farming pigs.

4.8 RECOMMENDATION

It is recommended that diets that contain 10% CP can be used in Kolbroek boars without any adverse effects on reproduction.

4.9 ACKNOWLEDGMENTS

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Chapter 5

TESTICULAR MORPHOLOGY AND HISTOLOGICAL CHANGES IN KOLBROEK BOARS FED DIFFERENT LEVELS OF PROTEIN DIET

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5.1 ABSTRACT

The objective of this study was to evaluate the effect of crude protein diet on testicular morphology of Kolbroek boars. A total of 14 Kolbroek boars with an average live mass of 98 kg were used. This was a continuous experiment as outlined in chapter 3 and 4. Two boars were selected from each treatment at the end of the protein digestibility. Selected boars were slaughtered and their reproductive organs were carefully dissected out and separated into different components and measured. The left and right testis were fixed with formaldehyde and later processed for histological assessment. There was no significant difference on length and weight for Kolbroek boars irrespective of the diet. However, there was a significant difference between 13 (3.5 mm) and 16 (5.0mm) protein on right testis width. Additionally, a relationship existed between the left and right testicular dimensions. There was a highly positive correlation between left testis length (LTL) and right testis length (RTL) (r=0.90). The left epididymis weight (LEW) and right epididymis weight (REW) showed also a highly positive correlation (r=0.69.

Negative correlations existed between body weight (BW) and RTL (r-0.78) also between BW and RTW (-0.05). The seminiferous tubules showed an active spermatogenesis with different tubules showing different stage of spermatozoa production. In conclusion, there was significant different on protein levels on testicular morphology of Kolbroek boars. In addition, testicular size estimated by external measurements good indicator of reproductive efficiency in boars.
Key words*:* Kolbroek, testicular morphology, histological pathology, seminiferous tubules, protein diet.

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5.2 INTRODUCTION

Testicular morphology is a major determinant of the reproductive performance, growth pattern and alternment of normal testis size in boars. Factors affecting reproductive performance in male animals include age, nutrition, environment, health status or disease, frequency of use, management and abnormalities (Brown, 1994; Oyeyemi & Okediran, 2007). Testicular growth performance may be an indicator of the reproductive status of boars (Umesiobi & Iloeje 1999). It has been reported that testis size and weight are correlated with daily sperm production and total sperm reserves (Huang & Johnson, 1996). Moreover, Rathje *et al*. (1995) reported that boars that had heavier testes did produce more sperm per gram of testicular tissue. Therefore, there is need to evaluate the effect of protein levels on testicular morphology of Kolbroek boars.

Testicular growth may be an indicator of the reproductive performance of the boars (Webb *et al*., 2006) and develop the testicular size at a young age (Mccoard *et al*., 2003). Testis size has been correlated with the sperm and testicular morphology (Umesiobi, 2006). Moreover, testicle size improve fertility and reproductive efficiency in boars (Huang *et al.,* 1996). Testicular steroid production is influenced by the development of the animal and at market weight, boars will vary in their sexual maturity (Haeussler *et al*., 2007).

Another aspect that determines testicular size and the number of sperm cells is the sertoli cells (McCoard *et al*., 2003; Franca *et al*., 2000). It has been reported that daily sperm production of boar increases with number of sertoli cells and testicular weight (Ford & Wise, 2009).

Furthermore, the maturation of leydig cells is reproduced by a testosterone between 35 and 38 days (Haeussler *et al.,* 2007). There is a need to evaluate the effect of protein levels on seminiferous tubules; testicular morphology and body weight of Kolbroek boars. Therefore, the objective of present study was to evaluate the effect of protein diet on testicular morphology in Kolbroek boars.

5.3 MATERIAL AND METHODS

The study was carried out at the Germplasm, Conservation and Reproductive, Biotechnologies (GCRB), and the Meat Science unit of the Agricultural Research Council (ARC), Animal Production Institute (API), Irene, Pretoria. The experimental pigs were carried for according to the guideline for the ARC-API. A total of 14 Kolbroek boars with a body weight of 98 kg were used and were randomly allocated to three levels of protein inclusion diets, Diet 1 (n=5); 2 (n=4) and 3 (n=5) comprising of 10, 13 and 16% protein; respectively. Two boars per treatment were slaughtered and their reproductive organs (testis and epididymides) were carefully removed and trimmed of adhering tissue. The boars were housed individually and given water *ad libitum*. The research protocol of the experiment was evaluated and approved by the Animal Ethics Committee of the ARC-Animal Production Institution (ref No. APIEC13/002), before the commencement of the experiment.

5.3.1 Testicular measurements

Two boars per treatment were slaughtered and their reproductive organs (testis and epididymides) were carefully removed and trimmed of adhering tissue. The left, right testis and epididymis were weighed using a sensitive electronic scale. Testicular and epididymal morphometric traits, left and right testis length and width were measured using Vernier calipers. The testicular volume was calculated using the following formula: volume = length 2 X width π/6 (Beckett *et al*., 1997). The skinfold thickness was carefully determined alongside the dimensional measurements with the Vanier calipers® and recorded.

5.3.2 Histological evaluation

The testes samples were weighed and fixed in 10% formaldehyde® before evaluation of leydig and sertoli cell, semiferous tube, germinal epithelium, spermatogonia, spermatids and primary spermatocytes. Sections of 4 mm were cut with a Leica sliding microtome (SM 2000R, Nussbach, Germany) and the slides were stained with haematoxylin–eosin. The average diameter of the tubuli was determined by planimetry. For characterization of the change of the leydig cell area, a total of five interstitial areas that were surrounded by three neighboring tubules were chosen at random and the number of leydig cell nuclei was counted. The numbers of leydig cells were not remarkably different between controls and immunized boars. The diameter of the nuclei and the diameter of the complete leydig cells were determined and the volumes calculated under the simplified assumption of a spherical shape of both cells and nuclei. Spermatogenic are characterizing into three sections activities of each testis were evaluated for the following spermatogenic cells: A-spermatogonia, B-spermatogonia, pachytene spermatocytes, round spermatids and elongated spermatids. For the quantitative distribution of spermatogenic cell types all sections were evaluated by the same person. Five representative round tubule for each of these eight stages were localized in the three sections and the cells were counted, so that the quantitative data for each cell type are based on 40 tubules in each boar. A photo-micrographic software - Phoenix Micro Image Analysis (2003) version 1.33 was used to project the slides on the computer for clear assessment.

5.4 STATISTICAL ANALYSIS

Data were analyzed using SAS, 1999. Student's t-Least Significant Difference (LSD) was calculated at the 5% level to compare treatment means of significant effects.

5.5 RESULTS

The effect of protein diet on testicular morphology of Kolbroek boars are in **Table 5.1**. There was no significant difference among the different protein diets on testicular morphology of

Kolbroek boars. However, the values tended to increase with the 16% protein diet. A significant difference was observed on the left epididymis weight of the Kolbroek boars fed different protein diets. According to table 5.1, there was no significant difference, were there was a significant different among the different protein diet on left epididymis weight 10 (29.2g), 13 (36.1g) and 16% (49.4g) and testicular volume (557.5, 407.4 and 708.5 cm3). Boars fed 16% of protein diet had greater testicular volume than 10 and 13% of protein diet though there is no significant different. Body weight and backfat thickness were not significantly affected by protein level. There was no significant different among protein levels on left epididymis weight. Although, there was no significantly different on 10, 13 and 16% of protein diet on right epididymis weight of Kolbroek boars.

Table 5.1 The effect of dietary crude protein levels on testicular weight and seminiferous tubule of Kolbroek boars (± LSD)

Parameter	**10% of protein diet (n=5)**	**13% of protein diet (n=4)**	**16% of protein diet (n=5)**
Body weight (kg)	83.0±21.2	99.0±1.4	95.0±7.1
Backfat thickness (mm)	19.5±0.7	20.0±1.4	19.5±0.7
Right testis length (mm)	7.1±1.5	6.5±0.7	7.1±0.1
Left testis length (mm)	7.6±0.8	7.5±0.7	8.0±0.0
Right testis width (mm)	4.0±0.0^{ab}	3.5±0.7^{b}	5.0±0.0^{c}
Left testis width (mm)	4.5±0.7	4.0±0.0	4.5±0.7
Right testis weight (g)	63.3±22.8^{a}	55.0±26.5^{b}	67.4±26.3^{a}
Left testis weight (g)	64.6±24.6^{a}	72.8±2.5^{b}	81.9±18.7^{c}
Right epididymis length (mm)	14.5±0.7	13.0±1.4	15±0.7
Left epididymis length (mm)	14.5±0.7	14.0±0.0	15.5±0.7
Right epididymis weight (g)	30.7±0.1	30.2±0.1	40.2±13.9
Left epididymis weight (g)	29.2±1.4^{a}	36.1±0.1^{b}	49.4±1.2^{c}
Testicular volume (cm^3)	557.5±176^{a}	407.4±35.6^{b}	708.5±108.5^{c}

$^{a, b, c}$ Means on the same row with different superscripts differ significantly P > 0.05).

Table 5.2 Pearson correlations coefficients among testicular traits, body weight and backfat thickness of Kolbroek boars

	RTL	RTW	RTW	LTL	LTW	LTW	REL	REW	LEL	LEW	BW	BF	TV
RTL	1.00												
RTW	0.08	1.00											
RTW	0.80	1.00	1.00										
LTL	0.90	0.20	0.80	1.00									
LTW	0.72	0.34	0.80	0.53	1.00								
LTW	0.64	0.30	0.74	0.70	0.80	1.00							
REL	0.74	0.24	0.83	0.72	0.71	0.40	1.00						
REW	0.20	0.54	0.60	0.30	0.64	0.70	0.50	1.00					
LEL	-0.08	0.80	0.25	0.12	0.32	0.21	0.45	-0.41	1.00				
LEW	0.12	0.71	0.20	0.46	0.17	0.60	0.16	0.69	0.60	1.00			
BW	-0.78	-0.05	-0.57	-0.42	-0.83	-0.45	-0.56	-0.10	0.17	0.28	1.00		
BF	0.51	-0.54	0.34	0.63	-0.16	0.11	0.22	-0.41	-0.50	-0.06	-0.03	1.00	
TV	0.63	0.78	0.58	0.60	0.83	0.70	0.67	0.69	0.60	0.58	-0.56	-0.23	1.00

RTL= right testis length (mm), RTW= right testis width (mm), RTW= right testis weight (g), LTL= left testis length (mm), LTW= left testis width (mm), LTW= left testis weight (g), REL= right epididymis length (mm), REW= right epididymis weight (g), LEL= left epididymis length (mm), LEW= left epididymis weight (g), BW= body weight (kg), BF= Backfat thickness (mm), TV= testicular volume (cm^3).

Pearson correlations coefficients among testicular traits, body weight, backfat thickness boars and backfat thickness of Kolbroek boars are summarized in Table 2. There was a highly positive correlation between LTL and RTL (r = 0.90). There was a highly correlated between RTW and RTL (r = 0.80). The LEW and REW showed also a highly positive correlation (r = 0.69. Negative correlations existed between BW and RTL (r= -0.78) also between BW and RTW (-0.05).

5.5.1 Histological morphology

The diameter of seminiferous tubules and volumetric proportions of spermatogenic elements of the boars were not significantly influenced by the different dietary protein treatments. There was no significant difference between 10, 13% and 16% of protein diet on leydig cells. This was due to of a numerous interstitial leydig cells that were visible in between seminiferous tubules and they have a normal appearance but on 10% protein interstitial leydig cells are prominent not increased above normal in relation to the other samples.

The seminiferous tubules all show active spermatogenesis, with different tubules showing different stage of sperm production. These results, suggest that seminiferous tubules, sertoli and leydig cells, spermatogonia, testicular morphology and spermatogenesis of Kolbroek boars were not affected by inclusion of different protein level. However, some tubules clearly showed the presence of numerous elongated spermatids and spermatozoa attached to the sustentacular cells and also loose in the lumen in 10% of protein diet. Varying degree of the spermatogenic cycle are visible in these samples and only in left testicles and right testicles in 10% of protein is extensive inactivity of the spermatogenetic tissue present and associated with some granulomatous inflammatory lesions. Although, 13% and 16% of protein diet also showed less active seminiferous tubules, there are interspersed by more active tubules showing all different stages of the spermatogenic cycle. There was no significant different on 10, 13 and 16% of protein in the left and right testicles. In the left testis found scattered tubules contain spermatozoa

loosely in the lumen as well, in the right testis found testicles scattered tubules show the presence of all levels/cylical stage of spermatogenesis in the right testicles.

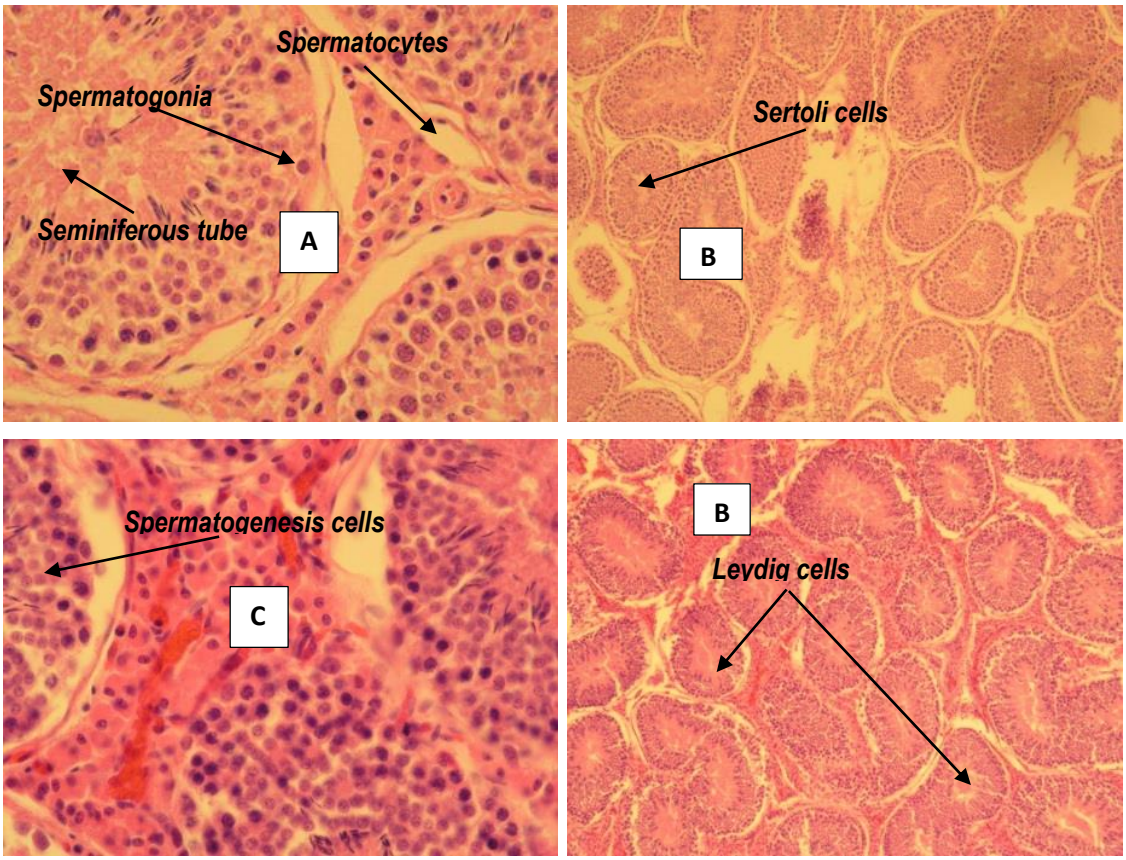

Figure 5.1 Histological structure of testis of Kolbroek boars fed different levels of protein, A= 10%, B=13% and C=16% protein

5.6 DISCUSSION

It was evident from this study that testes weights appeared not to be influenced by dietary protein levels. Furthermore, there is a clear indication of a strong relationship between the growth of testicular morphology, testis weight and body weight in boars. In this experiment the seminiferous tubules, serotonin and leydig cells, spermatogonia, testicular morphology and spermatogenesis of Kolbroek boars were not affected by protein levels. There were no relationship between left testis lengths, right testis weight, left testis width and right testis width of Kolbroek boars. Conversely, a negative correlation existed between body weight and testicles.

According to Kluber III *et al*. (1998), boars fed altrenogest had a greater volume percentage of lumen of seminiferous tubules than control boars. In addition, the control diet had a greater volume percentage of interstitial. Seminiferous tubule elongation occurs in response to sertoli cell proliferation in several species including boars (Kosco *et al*., 1990). The testis index in indigenous Chinese boars fed 250 ppm isoflavones was higher than in the control diet, while there was no difference between boars fed the control of corn based with 125, 125 ppm isoflavones or diethylstilbestero (Yuan *et al*., 2012). There was a significant difference between 10, 13 and 16% protein diet on left epididymis weight of Kolbroek boars.

The result showed that there were no relationship between testicular weight and body weight and back fat (Franca *et al.,* 2000) indicated that testicular weight was highly and significantly correlated with the body weight. The total length of the seminiferous tubule correlated significantly with the testicular weight and tubular diameter (Franca *et al.,* 2000). There was no relationship between epididymis and body weight of Kolbroek boars. This is similar to report by Schinckel *et al.,* (1991). There was a positive correlation between back fat thickness and testicles size of Klobroek boars. The According to Ugwu *et al.,* (2009) and Schinckel *et al*., (1991) reported that there is a positive correlations between testis size and backfat thickness does exists.

5.7 CONCLUSION

Results of this study suggested that there was a significant difference between 10, 13 and 16% levels of dietary protein inclusion on testicular morphology of Kolbroek boars. However, boars fed 16% protein diet had greater testicular weight than 10 and 13% protein diet. The result of the present study indicated that testicular measurements can be used to estimate the reproductive performance of boars. Therefore, the results suggest that testicular size estimated by external measurements is a good indicator of the reproductive status of boars, and boars with higher body weight and testis weight may produce more spermatozoa.

5.8 RECOMENDATION

Testicular morphology of boars was not affected by either 13 or 16% levels of dietary protein. Therefore, 10 % protein inclusion is recommended to be used for ration formulation of diets for Kolbroek boars.

5.9 ACKNOWLEDGMENTS

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Chapter 6

EFFECTS OF DIFFERENT DIETARY PROTEIN INCLUSION LEVELS ON NITROGEN BALANCE, NUTRITIONAL DIGESTIBILITY AND CARCASS QUALITY OF KOLBROEK BOARS

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6.1 ABSTRACT

The objective of this study was to evaluate the effect of dietary protein concentration on nitrogen balance, nutritional digestibility and carcass quality of Kolbroek boars. A total of 14 Kolbroek pigs boars that were selected at day old and were used previously for some experiments, were subsequently selected at an average weight of 98kg and randomly allocated to three protein diets comprising of 10, 13 and 16%. Faeces and urine were collected twice daily, and analysed for protein digestibility. At the end of the trial, two

Kolbroek boars per treatment were slaughtered. Carcass characteristics were measured and recorded. Drop loss was calculated as the warm carcass weight less the cold carcass weight. Dressing percentage calculations were determined as the cold carcass weight as a percentage of live weight. The head, kidneys and tail were then removed from the carcass. Data were analysed using Procedure General Linear Model. No significant difference was observed on protein digestibility, irrespective of the protein diet. Kolbroek boars were fed 16% protein; their digestibility was numerically higher (83.6 %) than 10 and 13% of protein diet with (82.5 %) and 10% protein diets (82.9%). There was no significant difference on 10, 13 and 16 % in visceral organs of Kolbroek. Water-holding capacity was not affected by different inclusion of protein levels for 10, 13 and 16 %, respectively. Body weight and carcass characteristics were not affected by dietary protein. However, boars at 13% protein diet were slightly heavier. There were no significant different between protein levels on carcass fat content , percentage carcass lean, leaf fat content, or dressing percentage of 10; 13 & 16% protein levels, respectively. There was no significant difference between protein levels on carcass characteristics in Kolbroek boars. In conclusion, there was no effect of different protein levels on Kolbroek boar carcass characteristics except on loin area. However 13% protein level had higher lion area (70.4 cm2) compare to 10 (53.3cm2) and 16% (64.6cm2). It is suggested that feeding Kolbroek boars with 13% protein level result in better quality of meat.

***Keywords*:** meat quality, indigenous pigs, feed levels, carcass characteristic.

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6.2 INTRODUCTION

Indigenous pigs are typical fatty pig breeds with 28 to 35% of meat quality (Chinnamani *et al.,* 2008; Egerszegi *et al.,* 2003). Nowadays due to economic reasons and customer demands, pig producers raise animals with carcass of acceptable quality and a minimum of excess fat (Chinnamani *et al*., 2008). Meat quality has become a primary focus for pig production (Newcom *et al.,* 2004). The mutton, beef and chicken meat alone cannot meet the animal protein requirements of the growing population (Chinnamani *et al.,* 2008).

There is a shortage of animal protein consumption due to the high cost of conventional sources of meat like cattle, pig, goat, sheep and poultry (Tewe, 1999). Indigenous pigs have been recommended as a good alternative source of cheap and high quality animal protein (Ironkwe & Amefule, 2008).

Nutrition has been reported to influence the quantity and percentage of muscle and fat tissue in carcass traits of Black Slavonian pig (Butko *et al*., 2007). Chinnamani *et al*., (2008) indicated that increasing the dietary crude protein level results in less fat deposition in the carcass of pigs. Furthermore, information regarding the effect of protein level on growth performance and carcass characteristics in the growing boar is somewhat limited and the results of studies to date are unpredictable (Sundrum *et al*., 2000). Protein represents the level at which amino acids are required for maintenance and body protein accretion in growing pigs (Tuitoek *et al.,* 1997). It is important to determine the effects of this fraction on both the digestive and metabolic utilization of energy in pigs (Sundrum *et al.,* 2000). Several reports have indicated that indigenous pig breeds can utilize fibre better than exotic breeds (Fevrier *et al.,* 1992; Kanengoni *et al.,* 2002; Ndindana *et al.,* 2002), especially with diets that are very high in fibre. Protein mass in animal can be determined analytically, using information on daily changes in nitrogen status and nitrogen balance (Tuitoek *et al.,* 1997).

Productivity and nutritional digestibility status are relatively unknown Indigenous pig traits (Campell *et al.,* 1990). Little information is available with regards to performance traits and digestibility coefficients of indigenous pigs (Lemus *et al.,* 2003). It has been suggested that the gene potential of ndigenous pigs (Campell *et al.,* 1990), could be utilized in metabolic studies related to obesity (Lemus *et al*., 2003). Chen *et al.* 1999; Devine *et al.,* (2005) indicated that growing pigs reduced their performance and excessive dietary protein levels, due to the metabolic cost of increased nitrogen excretion. Pigs diets formulated on an ideal protein, amino acids are provided in the proportions and for maintenance and protein accretion (Lemus *et al.,* 2003). There is a little information regarding the amounts of amino acids in the protein that are available to the animal for metabolism (Campell *et al.*, 1990).

Protein accretion of an animal depends on the rate of protein synthesis exceeding that of protein breakdown (Lemus *et al.,* 2003). Protein accretion is both processes of protein synthesis and breakdown which continue throughout life, even when growth is zero or negative Campell *et al*., 1990). The protein synthesis in the tissues of pigs increases and thus tend to change the growing pattern of pigs (Campell *et al.,* 1990). Protein metabolism is the processes that regulate protein digestion, amino acid metabolism and body protein accretion (Devine *et al.,* 2005). The objective of this study was to evaluate the effect of carcass quality, nitrogen balance and nutritional digestibility on Kolbroek boars.

6.3 MATERIALS AND METHODS

6.3.1 Experimental site

The study was conducted at the Agricultural Research Council (ARC) - Germplasm, Conservation & Reproductive, Biotechnologies (GCRB) and Meat Science units, South Africa. The ARC-Irene campus is located at 25° 55' South; 28° 12' East and located in the Highveld region of RSA and situated at an altitude of 1525m above sea levels (Webb *et al*., 2004). The protocol of the experiment was evaluated and approved by the Animal Ethics Committee of the ARC-API before the experiment could be initiated (APIEC13/002).

6.3.2 Experimental animals

A total of fourteen Kolbroek boars of at day old (1) aged, with a body weight of 98 kg from previous trail of testicular morphology and histological changes were allocated into three protein levels (10, 13 and 16%). The boars were housed individually and fed 1.5 kg per day and water *ad libitum*. At the end of experiment two boars per treatment were slaughtered. The slaughtering methods were approved by the Ethics Committee of the ARC.

6.3.3 Experimental diets

Diets were formulated to meet National Research Council (NRC) requirements (1998) using Format® international feed formulation software solutions (Table 6.1).

Table 6.1 Composition of experimental diets fed to growing Kolbroek pigs.

Ingredients kg/ton	10% protein diet	13% protein diet	16% protein diet
Wheat bran	151.3	155.4	159.4
Hominy chop	300.0	325.2	350.5
Maize meal	495.7	399.0	304.1
Soya oil cake	0.0	0.0	121.4
Monocalcium	25.0	16.5	7.9
Full Fat Soya Micro	10.0	20.0	30.0
Limestone	6.0	9.5	13.0
Salt	1.0	1.0	1.0
L Lysine HCL	6.0	6.9	7.8
DL Methionine	1.0	1.0	1.0
[1]Pig Supplement	4.0	4.0	4.0
TOTAL	1000	938.5	1000.4
Calculated composition			
Protein (%)	10.5	10.53	12.0
Energy/MJ/KG DM	17.0	17.71	18.0
Fat (%)	4.5	4.67	5.5
Phosphorus (%)	1.1	0.80	0.8
Neutral Detergent fibre (%)	30.4	30.00	30.0
Acid Detergent Fibre (%)	6.5	6.00	6.7
Calcium (%)	0.9	0.9	0.9

[1]The pig supplement contained vitamin A 6500000 iu; D3 1200000 IU; E 40000 IU; K3 2 g; B1 1.5 g; B2 4.5 g; B12 0.03 g; B6 2.5 g; Niacin 25 g; Calcium Pantothenate 12 g; Choline 190.5 g; Folic acid 0.6 g; Biotin 0.05 mg; Manganese 40 g; Zinc 100 g; Copper 125 g; Iodine 1 g; Ferrous 100 g and Selenium 0.3 g.

6.3.4 Carcass measurements

After slaughtering, carcasses were weighed soon thereafter to get the warm carcass weights. After 24 hours after being chilled at 2˚C, the cold carcass weights were obtained. Drop loss was calculated as the warm carcass weight less the cold carcass weight. Dressing percentage calculations were determined as the cold carcass weight as a percentage of live weight. The visceral organs were measured. The back fat and eye loin muscle area measurements were then taken from the carcass meat. Back-fat thickness and muscle depth were determined using Vernier caliper. Back-fat thickness measurements were made between the 2^{nd} and 3^{rd} rib. To have access to the loin for the measurements of the eye muscle length, eye muscle width and eye muscle-fat on the carcass. The lean meat percentage was calculated using the standardized formula (% lean=72.5114-0.4618V + 0.0547S).

6.3.5 Digestibility measurements

Digestibility was measured using the total collection method. Faeces and urine were collected once daily, pooled, and stored at −20°C until analyses for digestibility. The Kolbroek boar were allowed a 5-day acclimatization period, followed by a 5-day collection period. Urine was collected daily in buckets and acidified by the addition of 50 ml H2SO4 (25% v/v) so as to minimize the atmospheric loss of nitrogen. Each metabolism crate had a collection tray for urine collection and a fine-mesh plastic net just above the tray for faecal collection. In addition, glass wool was placed in the funnel of the collection trays to trap any faeces not retained by the net. Faeces were collected once and stored in plastic zip-lock bags to minimize nitrogen loss as ammonia. After the 24-h collection, faeces were weighed and stored at -20°C until required for analysis. The protein (N × 6.25) concentration in feed the nitrogen concentration in the faeces was were analysed by the macro-Kjeldahl technique using a Buchii distillation apparatus (Lee *et al.,* 2005).

6.3.6 Laboratory analysis

A representative sample of the diet that had been ground through a 1 mm sieve was used for analysis. Dry matter (DM), crude protein (CP) and crude fibre (CF) were determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 1995). DM was analysed by drying a 2 g sample at 105 C for 5 hours. To determine CF, a 2g sample was chemically digested and solubilised with dilute sulphuric acid and sodium hydroxide. To determine neutral detergent fibre (NDF), a 2 g sample was chemically digested and solubilised with dilute sulphuric and sodium hydroxide. The remaining fibre was then corrected for ash. The Kjeldahl method was used to determine the nitrogen content in samples (faeces and urine) and then the nitrogen content was multiplied by 6.25 for conversion into CP. Acid detergent fibre (ADF) were analysed by the methods of van Soest (1963). NDF was determined by extracting a 1 g sample in a neutral detergent solution and alpha amylase. NDF was determined after incubating a 1 sample with pepsin under acidic environment for 24 hours and then extracting the sample with an acidic solution , the remaining residue was then dried and ashed (Van Soest, 1963).

6.4 DATA ANALYSIS

Data were analyzed using Procedure General Linear Model. The significance difference between means was compared by Students t-test Least Significant Different (LSD). Differences with a probability of values $P>0.05$ were considered not significant. The data were analysed as a three treatments in a repeated change over design with diet. Student's t-Least Significant Difference (LSD) was calculated at the 5% level to compare treatment means of significant effects.

6.5 RESULTS

The effect of feeding pigs with diets containing different protein levels on the warm and cold carcass, lean meat percentage, loin area, chop and water holding capacity

characteristics of Kolbroek boars are presented in Table 6.2 There was no significant difference between protein levels on carcass feed conversion ratio in Kolbroek boars. Moreover, no significant difference was observed between protein level and body weight and carcass characteristics of Kolbroek boars irrespective of different protein levels (10, 13 or 16%).

Table 6.2 The effect of dietary protein levels on carcass characteristics and composition in Koelbroek pigs.

Parameter	**10% of protein (n=5)**	**13% of protein diet (n=4)**	**16% of protein diet (n=5)**
Initial weight	12.1±3.4[a]	13.4±2.6[a]	12.4±3.6[a]
Body (Kg)	83.0±2.2[a]	99.0±1.4[a]	95.0±7.1[a]
P2 live	19.5±0.7[a]	19.5±0.7[a]	19.5±0.7[a]
CL (cm)	79.5±13.4[a]	89.0±2.8[a]	91.5±0.7[a]
WC (kg)	69.7±16.4[a]	76.3±5.3[a]	75.2±1.8[a]
Loin area (cm^2)	53.3±2.2[b]	70.4±0.3[a]	64.6±8.2[ab]
CC (kg)	67.5±16.4[a]	74.7±5.0[a]	73.7±1.8[a]
Marbling mass	1.5±0.7 [a]	2.0±1.4[a]	2.5±0.7[a]
Chop mm	0.6±0.4[a]	0.5±0.1[a]	0.6±0.3[a]
Fat depth (mm)	52.5±3.5[a]	50±7.6[a]	51.9±2.6[a]
Lean meat (%)	54.5±3.5[a]	52.0±2.8[a]	56.0±1.4[a]
Dressing (%)	83.9±1.6[a]	77.0±4.2[a]	79.1±4.3[a]
BF last rib	65.3±6.7[a]	67.9±10.4[a]	65.4±7.0[a]
Water holding	0.4±0.0[a]	0.4±0.1[a]	0.3±0.1[a]
Colour *L*	34.2±2.4[a]	36.8±1.7[a]	37.7±2.3[a]
A	7.0±1.4[a]	4.9±11[a]	6.0±0.1[a]
B	3.3±2.8[a]	3.3±0.8[a]	4.6±1.4[a]
CDG	69.7±6.5[a]	76.0±5.7[a]	74.5±2.1[a]
Drip loss (%)	0.0±0.0[a]	0.0±0.0[a]	0.1±0.1[a]
Eye Muscle Area	2730.00± 32.52[a]	3849.50±1011.87[a]	2373.50±383.96[a]

[a-b] Values with different superscript differs significantly (P<0.05) L* (lightness), a* (redness), and b* (yellowness), CDG = carcass daily gain =,CL =carcass length , CC = cold carcass

Carcass fat content, percentage carcass lean, leaf fat content or dressing percentage were not affected by different protein inclusion. The effect of protein levels on the visceral organ characteristics of Kolbroek boars is presented in Table 2. Visceral organs and kidney was not affected by different protein diet of Kolbroek (P>0.05). Water-holding capacity was not affected by different inclusion. Body weight and carcass characteristics

were not affected by dietary protein (P>0.05). However 13 % Protein levels had higher lion area (70.4 cm2) compare to 10 (53.3 cm2) band 16 (64.6 cm2). It is suggested that feeding Kolbroek boars with 13 % protein levels results in better quality meat.

Table 6.3 The effect of different protein levels on the visceral organ characteristics of Kolbroek boars.

Parameter	**10% protein diet (n=5)**	**13% protein diet (n=4)**	**16% protein diet (n=5)**
Heart (g/Kg)	0.2±0.0	0.2±0.0	0.2±0.0
Pancreas (g/Kg)	0.1±0.0	0.1±0.0	0.1±0.0
Lungs (g/Kg)	0.2±0.1	0.9±0.2	0.8±0.4
Liver (g/Kg)	0.8±0.1	1.0±0.0	0.9±0.1
Small intestine (g/Kg)	1.2±0.1	1.8±0.4	1.7±0.3
Large intestine (g/Kg)	2.0±0.5	3.3±1.2	2.7±0.1
Stomach (g/Kg)	0.4±0.1	0.8±0.2	0.7±0.1

P > 0.05

Effect of protein levels on Energy, Fat, NDF, Ash, ADF and DM digestibility in growing Kolbroek pigs are summarized in Table 2. No significant difference was observed on protein digestibility, irrespective of the protein diet. Kolbroek boars were fed 16% protein; their digestibility was numerically higher (83.6 %) than 10 and 13% of protein diet with (82.5 %) and 10% protein diets (82.9%). There was no significance difference in digestibility of energy, fat, NDF, Ash, ADF and DM in Kolbroek boars. However, dry matter digestibility was numerically higher in pigs fed 16% protein diet (82.0%).

Table 6.4 Effect of experimental diets on nutrient digestibility in Kolbroek pigs (±LSD).

Parameter	10% protein diet (n=6)	13% protein diets (n=6)	16% protein diet (n=6)
Protein (%)	73±0.6	71±1.4	71±0.6
Energy (%)	91.2±0.1	91.4±1.2	93.0±1.0
Fat (%)	90.5±1.1	91.0±1.0	92.8±1.2
Neutral Detergent fibre (%)	65.0±0.3	67.3±0.3	65.2±0.4
Ash (%)	6.7±4.0	6.3±2.2	10.0±2.2
Acid Detergent Fibre (%)	43.7±2.6	44.8±8.3	45.3±4.4
Dry Matter (%)	79.8±0.5	80.2±0.3	82.0±0.5

LP= low protein diet and HP= high protein diets, Kg= kilogram, and G=grams

No significant effect was observed.

Table 6.4 Effects of dietary protein (mean (±SD) on Nitrogen balance and protein utilisation and protein accretion in growing pigs

Nitrogen balance	10% protein diet (n=6)	13% protein diets (n=6)	16% protein diet (n=6)
Nitrogen absorbed g/d	73±0.6	471±1.4	671±0.6
Nitrogen intake g/d	16.6±0.2	17.0±0.0	17.0±0.2
Feacal nitrogen g/d	2.0±0.0	2.2±0.1	2.3±0.0
Nitrogen in urine g/d	3.5±0.0	3.7±0.0	4.0±0.0

g/d- grams per day and %- percentage

No significant effect was observed.

The effects of dietary protein levels on nitrogen balance in growing pigs are summarized in Table 3. No significant difference was observed on nitrogen balance, irrespectively of the treatment. Nitrogen absorption, expressed as a percentage of nitrogen intake was increased in pigs fed 13 (17.0g/d) and 16% (17.0g/d) protein diet. Faecal and nitrogen in urine progressively increased as the levels of protein increased.

6.6 DISCUSSION

This study demonstrated that there was no significant difference on 10, 13 and 16 % in carcass characteristic and visceral organs of Kolbroek boars. Pigs fed low protein diets have been shown to have fatter carcasses compared with those fed high protein diets (Kerr & Easter, 1995; Tuitoek *et al*., 1997). The findings are in line with the results reported by Chen *et al*. (1995) that the weight of liver, pancreas, stomach, kidney and small intestine were not significantly different. There were no significantly different between protein levels in liver, pancreas, stomach, small and large intestine of Kolbroek boars. There was no significant difference between protein levels on carcass feed conversion ratio in Kolbroek boars. Also, There were no significant differences between the protein levels on the carcass characteristics. According to Heyer & Lebret (2007), the initial body weight did not influence carcass traits at Large White 70 and Large White 110 and did not modify the effects of feeding strategy on carcass traits.

There was no significant difference in the dressing percentage, in agreement with previous report on Nigerian indigenous, Duroc, Landrace and Large White pig breeds (Senne *et al*., 2000). There were no effects of dietary treatment on the composition of lean and loin meat (Kim *et al.* (2009). Fevrier *et al.* (1992) indicated that carcass characteristics were not affected by fibre levels in the diet. Kim *et al.* (2009) reported no differences in live weight, carcass weight, average daily gain and back fat-thickness between conventional and feeding in the fatty acid composition of meat quality traits of organically reared Korean Native black pigs.

There was no significant affected between 10, 13 and 16% protein diets on nutritional digestibility of Kolbroek boars. Kolbroek boars were fed 16% protein, their protein was

numerically higher (83.6%) than 10% (82.5%) & 16% (82.9g/d). Moreover, there was no significant difference 10, 13 and 16% protein diet on nitrogen balance of Kolbroek boars. The results showed that there was no significant effect on nutrient digestibility of Kolbroek boars, irrespectively of the protein diet. Similarly, Amaeafule *et al.,* (2009) indicated that there were no significant differences among the test ingredients in digestibility of DM, OM, CP, CF, nitrogen free extract and energy. The digestibility of DM, protein and energy decreased as NDF increased in Kolbroek pigs. The digestibility of dry matter, crude protein and energy decreased as total digestible fibre increased (Zhang *et al*., 2013). It was indicated that DM, N, Ca & P digestibility was not significantly affected by different protein diets (Yoo *et al.,* 2009; Wang *et al.,* 2002; Senne *et al.,* 2000; Gómez *et al*., 2002).

The results of this study showed that there were no significant differences on faecal excretion on Kolbroek pigs, irrespective of protein diet. No significant difference was noted between protein levels; however, it was observed that faecal excretion increased with increased levels of dietary protein inclusions (Kerr & Easter, 1995). Intake of nitrogen, faecal excretion of nitrogen, and total excretion of nitrogen were not affected by dietary treatment (Senne *et al.,* 2000). There was no difference in urinary nitrogen excretion between pigs fed the un-supplemented 12% protein diet and pigs fed the 16% protein diet (Amaefule *et al.,* (2009). Similarly Kerr & Easter, (1995) indicated that faecal nitrogen and energy excretion were not affected by dietary treatment when the by 12 and 16% protein diets.

6.7 CONCLUSION

In conclusion there were no effect of different protein inclusion levels on carcass characteristics, nitrogen balance and nutrition digestibility in Kolbroek boar, except on lion area. However 13% protein levels had higher lion area compare to 10 and 16% protein, respectively. It is therefore, suggested that feeding Kolbroek boars with 13% protein resulted in a better quality of meat.

6.8 RECOMMENDATION

Kolbroek boars may be fed different levels (10, 13 & 16%) protein diet. However, 10% protein diet was favourable since it was cheaper than 13 & 16% protein diet and the results (10%) were comparable to the other treatments (13 and 16%).

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Chapter 7

CHAPTER 7: GENERAL CONCLUSION AND RECOMMENDATIONS

7.1 GENERAL CONCLUSION

The different levels of dietary protein inclusions into pig diets had no significant effect on growth performance, age of attainment of puberty, hormonal concentration and libido status of Kolbroek boars. A strong correlation existed between ADG and FI. Negative correlation existed between ADG and FCR as well as FCR and FI. This is the first study that provided more information on growth performance and correlations that existed between growth performance parameters in Kolbroek boars. However, further studies are required to reduce fat content of Kolbroek boars. From the result of the present study, testicular measurements may be used to estimate the reproductive performance of boars.

7.2 GENERAL RECOMMENDATION

- The study proved that there is no need to increase dietary CP above 10%, since all levels of CP have similar effects on the growth performance, reproductive traits, testicular morphology, carcass characteristics and nitrogen balance.
- Further studies are recommended do reduce the backfat content of Kolbroek boars.
- Determination of the exact level at which crude protein should be included in Kolbroek boar diets would be highly beneficial in improving the reproductive status of breeding sire, with a concomitant economic benefits to the pig industry.