THE EFFECT OF CRUDE PROTEIN AND CALCIUM INTAKE ON FERTILITY OF MALE BROILER BREEDERS

by

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Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

In Animal and Poultry Science
School of Agricultural, Earth and Environmental Sciences
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As the candidate's supervisor I have/have not approved this dissertation for submission

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ABSTRACT

Broiler parent stock are selected to ensure that good characteristics will be passed to the offspring but management of broiler breeder birds is different to that of broiler chickens. Feeding broiler breeders *ad libitum* can result in body weight-related consequences such as musculo-skeletal disorders and the inability to mate successfully during natural mating. There is an age-related decline in fertility observed in broiler breeder flocks after 40 weeks of age. Management techniques such as feed restriction and spiking have therefore been employed to prolong and maintain fertility of breeders with increasing age.

Feeding a female ration to male broiler breeders may negatively affect reproductive efficiency. The possibility that excess nutrients supplied to male broiler breeders influence fertility cannot be ignored because male fertility has a significant impact on overall flock fertility. Because the female broiler breeder has a higher requirement of protein and calcium, the effects of these on fertility of males fed a female ration are worth considering.

The objective of this experiment was therefore to assess the effects of feeding four different diets, keeping feed allocation constant to result in 2 levels of crude protein intake and 2 levels of calcium intake (HP: LC, LP: HC, HP: HC, LP: LC) on semen quality of male broiler breeders in the production phase.

An increase in crude protein intake significantly increased ($p<0.05$) body weight of male broiler breeders after 41 weeks of age with birds fed HP: LC, LP: HC and LP: LC being significantly different to males fed HP: HC. There was a significant CP x Ca intake interaction on male broiler breeder bodyweight after 52 WOA.

There was no significant effect of CP intake on sperm concentration and sperm mobility. Calcium intake significantly decreased sperm concentration at 42 and 60 WOA but had no effect on sperm mobility. There was no significant influence of CP intake on sperm concentration with age.

There was no significant response in overall log number of inner perivitelline layer sperm holes and across all ages. Log number of IPVL sperm holes declined with age from 37-59 WOA with day 2 post artificial insemination having a higher number of IPVL sperm holes counted across all ages.

PREFACE

The experimental work described in this dissertation was carried out in the school of Agriculture, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, from February 2014 to December 2015, under the supervision of Dr N.C Tyler and Dr M. Ciacciariello.

This study represent original work done by the author and has not been submitted in any form for any degree or diploma to any other tertiary institution. Use of other author's work has been duly acknowledged in the text.

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DECLARATIONS

1. The research reported in this dissertation, except where otherwise indicated, is my original research

2. This dissertation has not been submitted for any degree or examination at any other University.

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ACKNOWLEDGEMENTS

To Dr N.C Tyler, thank you for the assistance and patience. Your guidance, time and attention throughout the study is sincerely appreciated.

To Dr M. Ciacciariello, Thank you for your availability, motivation and endless input.

To the College of Agriculture, Engineering and Science, thank you for the College bursary that sponsored the experimental work, accommodation and subsistence

My gratitude to National Chicks Pty Ltd for the provision of male broiler breeders for my trial

My sincere gratitude to Ukulinga research farm staff, the department's technical staff, I would not have survived my experimental work without all of you

To my Parents and entire family, thank you for the continued support in everything I do, you have made me the person I am today and I will continue to make you proud

To my colleagues and close friends, the encouragement and support is sincerely appreciated

To God, Thank you for the blessings over my life and for always looking out for me. You are my strength like no other, thank you Father

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ABBREVIATIONS USED

BW - bodyweight

CP - crude Protein

Ca - Calcium

HP:LC - high protein and low calcium

LP:HC - low protein and high calcium

HP:HC - high protein and high calcium

LP:LC - low protein and low calcium

IPVL - inner perivitelline layer

OPVL - outer perivitelline layer

WOA - weeks of age

PAI - post-AI

µl - micro litre

nm - nanometer

lsd - least significant difference

sed - standard error of difference

ANOVA - analysis of variance

CHAPTER 1

GENERAL INTRODUCTION

The production of eggs in broiler breeder flocks is essential as it directly influences the profitability of the flock made possible by the contribution of both the male and female. Although it has been reported that females have a greater contribution to flock fertility than males (Gumulka & Kapkowska, 2005), it has also been argued that male broiler breeders have a significant contribution to fertility in broiler breeder flocks (Wolc *et al.*, 2009). It is for this reason that improvements in growth, carcass parameters and other meat yield traits come through the male-line but maintaining a balance between meat yield traits and reproductive traits is difficult as they are inversely related. Broiler breeder males are genetically selected for the traits they will pass on to their offspring and continued selection for these traits may be at the expense of the male's reproductive capability (Lesson & Summers, 2000; Romero-Sanchez *et al.*, 2007). Males selected for higher growth rate are more prone to excessive body weight (BW) gain which can result in reduced mounting ability, reducing the chances of achieving a successful copulation (McGary *et al.*, 2003).

Male broiler breeders therefore require proper management strategies during the growing period to control BW and maintain fertility. Structured feed restriction programmes have therefore been set in place in an effort to control the BW.

There is a significant reduction in fertility of broiler breeders observed after peak fertility, at about 30-40 weeks of age (Hocking, 1989; Bramwell *et al.*, 1996). One reason for this age-related fertility decline has been reported to be due to males becoming large and broad-breasted towards the end of the reproduction period, making it difficult for them to successfully achieve cloacal contact during mating (Hocking & Bernard, 1997b). In addition, the decline in fertility has also been attributed to a decline in sperm quality such as semen concentration and volume (Sexton *et al.*, 1989), and a significant decline in mating activity. Hocking & Duff (1989) suggested that the decline in mating activity was a result of aging broiler breeder males becoming more susceptible to musculo-skeletal disorders which reduce mating efficiency. Strategies such as artificial insemination, feed restriction and spiking are possible management strategies to mitigate the age-related decline in fertility and to maximise reproductive efficiency of male broiler breeders to ensure consistent flock fertility with age.

The nutrition of broiler breeder males is often overlooked and they are often fed a female ration which consists of a higher nutrient content than that required, due to the impracticality of feeding a separate ration. Crude protein and calcium are two important nutrients for egg production and hence they are required in higher quantities in egg-laying hens than in males. Overfeeding of these nutrients, besides the cost implication, may even result in negative effects on male broiler breeder fertility through an interaction or separately (Fontana *et al.*, 1990; Hocking, 1990; Hocking & Bernard, 1997a; Borges *et al.*, 2006; Tyler & Bekker, 2012). There is however inconsistency on the reports in effects of feeding males a female ration on male reproductive efficiency and this may be due to the inaccuracy of the various measurement techniques used to gauge male fertility.

The aim of this experiment was therefore to determine the effects of feeding different levels of crude protein and calcium at 32 WOA till 61 WOA on the reproductive performance of broiler breeder males using sperm concentration, sperm mobility and sperm penetration through the perivitelline layer of eggs as measures of male reproductive potential.

CHAPTER 2

Literature review

2.1 **Introduction**

The goal in broiler breeder production is to maximise the number of hatching eggs. Genetic selection in broiler breeders has been focused on improving meat yield traits amongst other productive traits. There is however a negative correlation between meat yield traits and reproductive traits (Lesson & Summers (2000). Male and female broiler breeders have different nutrient requirements. The greater requirement for crude protein (CP) in female breeders is due to the cost of egg production, and since males are often fed a female ration, there has been a growing interest in whether the higher CP content of the diet has any effect on male fertility. There have been reports on the effects of dietary CP on male fertility where low CP rations (12-14% CP) have been found to have a positive effect on fertility in some instances (Wilson *et al.*, 1987a; Hocking, 1989) but no reports of a negative effect of low CP in others (Fontana *et al.*, 1990; Hocking & Bernard, 1997b). Female broiler breeders also have a greater requirement for calcium (Ca) and there is little work that has investigated the impact of increased calcium intake on male fertility.

This review will cover fertility of broiler breeders, with an emphasis on the decline in fertility late in the production phase and assess measures in place for reducing the decline in fertility while maximising efficiency. The influence of nutrition, including feeding practices, nutrient requirements of male broiler breeders and impact of different nutrient intakes (especially CP and Ca) on fertility will be discussed. This review will also evaluate different methods of assessing sperm quality.

2.2 **Male management through feeding**

There has been a growing interest in the nutrition of males and recommendations focus on implementation of strategic feed management programs during rearing and production. Body weight management in broiler breeders during the rearing stage is critical and can have lifelong effects on the reproductive efficiency throughout the production period (Romero-Sanchez *et al.*, 2007). Restricted feeding has therefore been implemented by commercial breeders from an early age at rearing and throughout the production phase in an attempt to control male BW, achieve the target BW at each age and prolong reproductive lifespan (Sahraei, 2012). Feeding a diet according to breeder recommendations is important to meet nutritional requirements and promote optimum performance provided that proper feeding management is implemented at all stages.

2.2.1 **Body weight control**

An appropriate feed restriction practise is used to control BW of males in rear and after sexual maturity with the goal of optimising reproductive performance. It is important to grow the parent stock to the breed-recommended target growth curve allowing both sexes to achieve optimum performance through BW control which prevents overweight birds that could result in impaired mating frequency, sperm mobility (Bilcik *et al.*, 2005) and overall reproductive performance (Hocking & Duff, 1989; Hocking, 1990; Romero-Sanchez *et al.*, 2007). Good BW control using feed restriction is beneficial through an increased egg production, hatchability, egg quality and fertility (McDaniel *et al.*, 1981). To achieve flock uniformity and adherence to the breeder recommended growth curve, feed management through feed restriction is an important management factor.

2.2.2 **Feed restriction vs *ad libitum* feeding**

Feed restriction is essential in controlling the BW of the breeder flock to maintain the appropriate fertility required for a commercial breeder flock (Brown & McCartney, 1983). Hocking (1990) reported a decreased fertilising ability from broiler breeder males below the standard BW when compared to males that follow the bodyweight curve.

Duncan *et al.* (1990) reported that feed restriction in the adult male breeder has the potential to lessen the age-related decline in mating frequency by preventing excessive BW gain. High reproductive performance (sperm concentration, semen volume and total sperm yield) and low mortality was reported when breeder males were subjected to restrictions of about 85% of the breeder recommendations (Revington *et al.*, 1991). Brown & McCartney (1986) reported better reproductive performance in males subjected to feed restriction while Brown & McCartney (1983) reported no significant effect on fertility or hatchability measured from eggs of artificially inseminated female breeders.

The conflicting results show that the degree of restriction needs to be understood clearly in the context of nutrient requirements as restriction changes with improvements in growth selection over time. Although restricting males is essential to improve fertility, severe restriction in males with improved genetic potential negatively affects fertility through a negative influence on growth rate and a decline in the percentage of males producing semen (Cerolini *et al.*, 1995). Although Brown & McCartney (1986) at 55% restriction of the recommended intake reported a reduced semen volume, semen-packed cell volume and testis weight, Duncan *et al.* (1990) observed a decline in sperm quality, libido and fertility of severely restricted males (67% of recommended intake)

between 38 and 58 weeks of age. Similarly, Cerolini *et al.* (1995) reported a decrease in the number of fertile eggs when males were severely restricted (110 g/bird/day).

Ad libitum feeding may be beneficial to spermatozoa production (Sexton *et al.*, 1989), but not to mating ability, due to an increased incidence of foot and leg disorders, difficulty in achieving cloacal contact during mating and therefore a decline in mating activity and efficiency (Hocking, 1989; McGary *et al.*, 2002; Romero-Sanchez *et al.*, 2007).

2.2.3 **Separate-sex feeding**

Separate feeding of females and males makes it possible to control feed allocation and to feed males a diet formulated specifically for male requirements. This is relatively easy during the rearing phase when males are reared separately to females and is achieved with the use of exclusion grids and feeder heights during production. The independent consideration of male and female nutrition improves the control of male BW and hence uniformity, which improves mating ability and therefore fertility (Hocking, 1990; NRC, 1994)

2.3 **Broiler breeder fertility**

In order to achieve maximum egg production, fertility is an important factor that needs to be enhanced through proper management suggested in breed-specific parent stock manuals. It is a common misconception that female breeders contribute more to fertility than males because they constitute the greatest proportion of the flock and produce eggs. Breeder males may represent a small percentage of the flock but they contribute equally to fertility in terms of the breeding value, making them just as important as female breeders. Wolc *et al.* (2009) reported broiler breeder

males to even be more important because each male has a greater influence on egg fertility than each female, determined from the high heritability and repeatability values for male effects compared to female effects.

2.3.1 **Decline of fertility with age**

Age has an adverse influence on the reproductive performance of broiler breeders. Fertility of male breeders reaches a peak at 30 to 40 weeks of age and then declines until the end of production with Bramwell *et al.* (1996) reporting a 5.7% decrease in fertility from 37 to 63 weeks of age. Even though fertility of naturally-mating birds drops after 40 weeks, Hocking (1989) concluded that it is only after 50 weeks that the effects become large due to more males losing weight. The decline in flock fertility was also reported to be predominantly a male problem (Brillard & McDaniel, 1986), where the drop in fertility has been attributed to the decline in sperm quality, semen concentration and volume (Sexton *et al.*, 1989; Hocking & Bernard, 1997b), and a decline in mating activity (Hocking & Duff, 1989). Revington *et al.* (1991) reported no differences in the percentage of male breeders producing semen at any age, suggesting that fertility decline with age is likely to be a semen quality factor rather than a decline in semen production. This is, however, contrary to Sexton *et al.* (1989), who reported a significant negative effect of age on sperm concentration and sperm yield. Mating activity decline is due to a reduction in mating frequency and efficiency which has been suggested to be related to poor feeding management of male breeders and also an increased susceptibility to musculo-skeletal lesions with age hence a significant impact in reducing fertility (Hocking & Duff, 1989).

Bramwell *et al.* (1996) reported no differences in fertility when semen from roosters aged between 39 and 63 weeks was collected and used to inseminate hens. Leg problems, lameness and libido-decline contribute to the decline in reproductive efficiency of naturally-mating birds by making it anatomically difficult to mate successfully (Duncan *et al.*, 1990; Hocking & Bernard, 2000; McGary *et al.*, 2002) hence artificial insemination in older males has been suggested as a way to minimise the drop in fertility (Brillard & McDaniel, 1986; Cerolini *et al.*, 1995; Romero-Sanchez *et al.*, 2008). The decline in fertility may also be related in part to the differing reproductive ability among males whereby certain males have higher fertility than others.

2.3.1.1 **Ways to minimise age-related fertility decline**

2.3.1.1.1 **Use of feed allocation**

Despite sufficient allocation of feed, the fertility of broiler breeder males decreases after 40 weeks of age (Hocking, 1989; Bramwell *et al.*, 1996; Lesson & Summers, 2000; Gumulka & Kapkowska, 2005). It has therefore been suggested by Romero-Sanchez *et al.* (2008) that increasing the feed allocated to broiler breeder males after 49 weeks of age counteracts the age-related fertility decline but this is dependent on the weight of the males relative to that of females because increasing the quantity of feed may result in males becoming too heavy and therefore damage females during copulation

When broiler breeder males were fed 91 g/bird/day (13.1% CP) there was a reduction in average semen volumes, proportion of males producing semen and testis weights at 50 weeks of age when compared to males to higher allocations (102, 113, 125 & 136 g/bird/day) and the sperm count per ejaculate from males of 30 to 50 weeks of age was lower for males given 91g/bird/day than those

given 136 g/bird/day of feed (Buckner *et al.*, 1986). Cerolini *et al.* (1995) found that supplying 110 g/bird/d of feed to broiler breeder males during the reproductive period depressed fertility, while that of 130 g/bird/d resulted in improved fertility. Hocking & Bernard (1997a), however, found no differences in fertility between males fed an increasing allocation and those under a fixed allocation (120 g/d) until the end of the production period, suggesting that there may be other factors contributing to the decline in fertility during the breeding phase. Minimising the fertility decline in the production phase also consists of important practical considerations in the management of broiler breeder males to maximise efficiency with age, some of which are mentioned below:

2.3.1.1.2 **Spiking**

Spiking is used in poultry to minimise the fertility decline in male breeders and maintain flock reproduction efficiency (Garmon & Hogan, 2008). Spiking is the introduction of young males (25-28 weeks old) to a 40-50 week broiler breeder flock in an effort to compensate for the age-related decline in fertility and this practiced has spread in various breeder operations (Brillard, 2004). When spiking is implemented it is said to result in significant increases in fertility visible 2-3 weeks post-spiking because of an increase in male to male competition for hens hence an increase in the mating frequency of old roosters (Casanovas, 2002). Bramwell *et al.* (1996) reported no significant improvement in fertility after spiking the flock with young males while in a more recent study, Chung *et al.* (2012) reported a significant increase in fertility of a spiked broiler breeder flock between 42-52 weeks.

Interestingly toward the end of production (54-58 weeks), there were no significant fertility differences between the spiked and control flock. This suggests that the positive effects of spiking may only last for a few weeks. There are concerns associated with the use of spiking and these involve the expense associated with running a separate house for the young males, replacement males being too young to successfully compete with older males hence delay their contribution to fertility and the biosecurity risk associated with the introduction of young males to an existing flock,

2.3.1.1.3 **Double inter-spiking**

As means to address the concerns of spiking, double inter-spiking was developed, which is the exchange of experienced males from two different houses regardless of the age of the male. The first spike is usually done between 40-50 weeks of age and the second spike is recommended when mating activity and fertility begin to decline around 50 weeks of age. This practice is believed to significantly improve fertility levels of the flock as unfamiliar roosters disrupt the flock pecking order/hierarchy thus increasing mating behaviour and sexual activity which compensates for the loss in libido and lack of mating interest with age (Casanovas, 2002). It was also concluded that double inter-spiking at 40 and 48 weeks of age stimulates a high mating activity and maintains significantly higher hatchability levels in broiler breeder flocks. There are less biosecurity risks associated with this practice and no cost involved as there are no extra males required. However, an increase in aggression between roosters in a double-inter-spiked flock is possible and may result in limited male to female interaction and more time being spent on male to male interaction which reduces mating activity.

2.4 **Sperm quality and measures of fertility**

The quality of sperm is an important factor in reproduction of male breeders and it varies with individual males and samples. Wilshart & Palmer (1986) concluded that the quality of sperm used to inseminate hens is a more limiting factor than the number of sperm inseminated in relation to egg fertility. The ability of the male to successfully mate and the quality of the semen produced are important factors in fertility. Methods used to assess reproductive performance of chickens have been limited to semen characteristics, libido, mating behaviour and conformation (Wilson *et al.*, 1979; Wilson *et al.*, 1987a; Hocking, 1989; Revington *et al.*, 1991). Sperm concentration, semen volume, testis weight and number of sperm per ejaculate have been historically used to assess broiler breeder fertility but through research developments, more reliable assays have been developed namely the sperm mobility assay (Froman & McLean, 1996) and counting the points of sperm hydrolysis in the perivitelline layer of eggs post-insemination/mating (Bramwell *et al.*, 1995; Gumulka & Kapkowska, 2005). Sperm quality is measured in different ways but not all these techniques represent the full fertilising capacity, which makes it difficult to assess the impacts of nutrition-related studies on male fertility when the more traditional methods of sperm quality have been used, which may not correlate well with fertilising ability. This section will review methods of assessing sperm quality, and the correlation with fertility.

2.4.1 **Semen characteristics**

2.4.1.1 **Semen volume and concentration**

Bilcik *et al.* (2005) reported a significant negative relationship between semen volume and semen concentration. However, volume is difficult to measure as this can vary depending on the time of

last ejaculation. The quantity of sperm produced by male breeders is not as important as sperm quality because spermatozoa is lost in the female oviducts as the sperm storage tubules limit the number of sperm stored, and the higher the quality of the sperm used to inseminate hens the higher the chances of storage in sperm storage tubules (SST) and hence successful fertilisation (Parker *et al.*, 2002). There is a significant correlation between the fertilising ability of males with measures of sperm motility, sperm ATP concentration and the morphological integrity of spermatozoa, but not with sperm concentration (Wilshart & Palmer, 1986). Average sperm concentration is reported to be 7 billion sperm per ejaculate and a maximum of 8.2 billion per ejaculate has been reported in Brown Leghorn males (Wilshart & Palmer, 1986), but Brillard (1993) recovered a maximum of 2.2 X 10^6 sperm in the SST of chickens which was 0.9% of the sperm number (250 X 10^6) used for insemination.

Although high sperm concentration is thought to be linked to fertility, males with a high sperm concentration in one study were reported to have significantly lower fertility, associated with a higher sperm cell mortality, resulting in failure of the sperm to reach the site of fertilisation (Bilcik *et al.*, 2005). However, reduced sperm concentration and volume with age contributed to a significant decline in fertility of broiler breeder males (Sexton *et al.*, 1989; Zhang *et al.*, 1999).

2.4.2 **Sperm penetration of the Perivitelline layer**

Sperm penetration analysis is an accurate method for gauging semen quality as it indicates the fertilising potential of sperm. Recent advances in assessing male broiler breeder fertility have led to several studies using the quantification of the number of sperm in the perivitelline layer of eggs

(Wishart, 1987; Brillard & Bakst, 1990; Wilshart, 1997; Tyler & Bekker, 2012; Holtzhausen, 2013) which is a direct result of the number of spermatozoa that were actually selected by the SST for storage (Brillard & Bakst, 1990; McGary *et al.*, 2002). Sperm storage tubules are essential in ensuring an increase in the probability of fertilisation following a single insemination, with more points of hydrolysis in the IPVL indicating more sperm stored in the SST thus contributing to higher fertility (Donoghue *et al.*, 1999). The IPVL sperm holes not only show egg fertility but also give information of the length of the fertile period (Gumulka & Kapkowska, 2005).

The sperm that penetrates the IPVL of laid eggs can be visualised and quantified through light microscopy (Bramwell *et al.*, 1995). The IPVL sperm holes are found concentrated around the germinal disc (Wishart, 1987). According to Bramwell (1998), an average sperm hole count of 30 per mm^2 or more around the germinal disc in a flock is necessary to maintain good fertility. Although it only takes a single sperm for one egg to be fertilised, Brillard & Antoine (1990) and Wishart (1987) reported that only 2.4 and 2 spermatozoa in a 5.5 mm^2 area respectively are necessary for a fertile chicken egg. Wilshart, (1997) reported that eggs had a 50% probability of being fertile when 3 or more sperm penetrated the IPVL and a peak fertility when 6 or more sperm around germinal disc penetrated the IPVL. McGary *et al.* (2002) found no sperm penetration through the perivitelline layer in most of the infertile eggs collected from a broiler breeder flock.

The duration of fertility reveals the reproductive capacity of a flock, which is significantly correlated with number of sperm penetration holes in the IPVL of eggs. The number of sperm in the IPVL of oviposited eggs decreases logarithmically with days after insemination and because of a correlation between fertility and number of sperm per unit area of membrane, the probability of eggs being fertile declines logarithmically with days after insemination (Wishart, 1987). Thus,

as the mating frequency of the flock declines with age, the chances of infertile eggs increase. The highest number of IPVL sperm holes are observed on the first few days post-insemination and decrease as days post-insemination increases (Fasenko *et al.*, 2009). This agrees with Bramwell *et al.* (1996), who reported that sperm holes in the IPVL of eggs following insemination decreased with an increase in days post-insemination.

2.4.3 **Sperm mobility**

Sperm mobility is a quantitative trait that plays an important role in the fertility of poultry species. Developed by Froman & McLean (1996), the sperm mobility assay measures the progressional movement of sperm into inert, dense and non-toxic diluents, such as Accudenz® solution (Donoghue *et al.*, 1999). Quantification of this trait is by overlaying a sample of semen on Accudenz® solution within a polystyrene curve, incubating the cuvette for 5 minutes at body temperature (41^{o}C) and measuring the sperm penetrating the Accudenz® layer at 550nm by a spectrophotometer (Froman & Feltman, 1998). The mobility assay imitates the physiological conditions of the reproductive tract making it possible to differentiate between male sperm as it is expected that low mobility sperm fails to penetrate the Accudenz® while mobile sperm penetrates rapidly.

Through observation of sperm hydrolysing the IPVL, Donoghue *et al.* (1999) reported a compromised ability to reach the site of fertilization by low mobility sperm while average and high sperm mobility males resulted in high number of hatched eggs in turkeys. This is supported by (Froman *et al.*, 2006), hens had to be inseminated with up to three times as many sperm when low

sperm mobility sperm was used, to maximise filling of the SST. Birkhead *et al.* (1999) reported a greater proportion of fertilised eggs from high-mobility males compared with average-mobility males. Froman *et al.* (2002) concluded that sperm mobility influenced the rate at which sperm were lost from SST, with highly mobile sperm being lost at a significantly slower rate resulting in a longer fertilising capacity. The mechanism by which high-mobility sperm succeeds in fertilisation is because they manage to enter the SST and penetrate the IPVL of eggs in greater quantities than low-mobility sperm (Birkhead *et al.*, 1999).

2.5 **Secondary Male traits**

Physical male characteristics have been used as an aid to management and as a tool to measure the reproductive performance of a rooster. The use of these traits is based on the fact that physical appearance in roosters is influenced by the production of testosterone. Testosterone produced by the testes and is believed to be associated with sexual behaviour of the male chickens. Secondary male characteristics such as comb size, area and colour are non-invasive measurements of fertility and testis size (McGary *et al.*, 2002).

2.5.1 **Comb size**

Comb size has been used as an indicator for the overall reproductive status of a rooster. It has also been used by females in selecting their mates as reproductive performance of a rooster is correlated to the size of the comb with Bilcik *et al.* (2005) and McGary *et al.* (2003) reporting a positive relationship between reproductive behaviour and comb area. It was further found that comb size and testicular weight were positively correlated (Tyler & Gous, 2008) indicating that male broiler breeders with large combs and/ or large testis are reproductively dominant in a flock. There is also

a direct correlation observed between testis size and the quantity of semen produced, semen volume and sperm concentration (Hocking & Duff, 1989; Lesson & Summers, 2000). Brown & McCartney (1983) however, found that breeder males with the largest testis at 54 weeks of age did not produce the largest amount of semen.

Testicular size is however difficult to assess as testis are situated internally and although it has never been done practically, possible visualization of the testis through ultrasound has been reported. Tyler & Gous (2008) reported that 69% of the variation in average testis weight was accounted for when comb area was used to predict average testis weight, therefore concluding that comb area provides a good non-invasive technique to measure testicular weight and therefore fertility. Although Wilson *et al.* (1979) earlier reported a non-significant correlation between fertility and comb size, McGary *et al.* (2003) concluded that secondary characteristics like comb size (area and length) are reliable indicators of fertility in broiler breeders. It is for this reason that hens may choose roosters with large combs as they are indicative of good fertility in a flock.

2.6 **Nutrient requirements and effects on fertility**

2.6.1 **Crude protein**

It is impossible to ignore CP in male fertility as it plays an integral role in reproduction efficiency of broiler breeders at all stages (Zhang *et al.*, 1999). Separate-sex feeding and changes in genetics have prompted the understanding of male nutritional requirements separately from females. Crude protein is an important nutrient for male broiler breeders as it plays a significant role in the growth of birds through BW management which impacts the reproductive performance of male and female broiler breeders (Duncan *et al.*, 1990). Throughout this review, where possible, crude protein

intake is reported in g of CP/bird/d but in studies where feed intake was not reported, CP% is used. Wilson *et al.* (1987a) reported that 12 or 14% CP could be given to male broiler breeders without affecting BW, semen volume and sperm concentration and that significantly more males in the 12 and 14% dietary CP diet produced semen from 27 to 30 weeks of age than those given higher levels of 16 or 18% CP. It has also been recommended that male broiler breeders be fed CP levels of 12.6% for maximum fertility (Tyler & Bekker, 2012).

In different studies the effects of CP on fertility of males was only observed after certain ages which may be due to the different levels of CP used. Hocking (1990) reported that giving naturally-mating males a high dietary CP of 16% during the breeding period results in a decline in fertility from 45-60 weeks of age compared to males given a lower protein level (11%). It was found that a lower protein intake of 10.8 g CP/bird/d could be given to male breeders on a further restricted basis compared to breeder recommendation-fed males with no detrimental effect on reproductive function (measured as semen volume, concentration and number of sperm per ejaculate) but a significant reduction in BW was observed as breeder recommendation-fed males were heavier than those given a 10.8 g CP/bird/d at isocaloric intakes (Wilson *et al.*, 1987b). Fontana *et al.* (1990) reported no significant differences in fertility between males fed different levels (12, 14 and 15%CP) which agrees with Hocking & Bernard (1997b) who also found no significant differences in fertility between breeder males given a high 16% and a low 12% CP feed.

2.6.2 **Calcium**

Calcium is an important mineral as it plays a role in skeleton formation, egg production and other biological functions. Feeding adequate levels of Ca is important for regulating sperm physiology,

sperm motility, metabolism and membrane function while it also has a role in sperm capacitation, acrosome reaction and fertilisation (Ren *et al.*, 2001; Kanyinji & Maeda, 2010). An excess supply of dietary Ca however interferes with the availability of other minerals, namely: phosphorus, zinc, manganese and magnesium (NRC, 1994) resulting in an increase in the requirement of these minerals. Male broiler breeders require Ca in lower quantities than females as they do not produce eggs but feeding males a diet formulated for female requirements does not seem have a negative effect on male broiler breeder performance (Fontana *et al.*, 1990; Hocking & Bernard, 1997b).

More than 4 decades ago, it was reported that the Ca requirement for male broiler breeders was below 0.27% but levels above that (3%) were also reported to not impair fertility (Wilson *et al.*, 1969). According to (NRC, 1994), broiler breeder males require 0.2 g Ca/bird/day from 20-60 weeks of age.

It has been reported that the quality of chicken sperm can be significantly improved through alterations in the diet (Bongalhardo *et al.*, 2009; Kanyinji & Maeda, 2010). Calcium levels in the diet possibly affect poultry sperm motility and this has been reported in a study done with Plymouth Rock roosters where additional dietary Ca (2% added Ca) increased calcium concentration in the seminal plasma which improved sperm quality measured as sperm motility (Kanyinji & Maeda, 2010). After feeding broiler breeder males levels of 0.5 -0.7 g Ca/bird/day, Kappleman *et al.* (1982) found no significant differences in reproductive performance (measured as semen volume, spermatozoa concentration and fertility of eggs hatched). Wong *et al.* (2001) found no significant correlation between blood and seminal Ca concentration, sperm concentration, motility and morphology. More recently, Rosa *et al.* (2010) concluded that Ca levels

of 0.90% in the diet were adequate for reproductive efficiency (measured using semen volume, number of sperm cells and egg fertility) of male breeders during the productive period.

Male chickens fed high dietary Ca levels exhibit low blood cholesterol (ch) levels which may result in the production of sperm with a high membrane integrity and thus an increased fertilising ability (Ditscheid *et al.*, 2005; Shende *et al.*, 2012) since sperm motility increases with decreasing blood cholesterol levels (Kanyinji & Maeda, 2010).

2.7 **Calcium and protein interaction**

Male broiler breeders fed a female diet are probably eating excess CP and Ca and these may influence overall flock performance in the reproduction phase as discussed above, but there may also be a combined effect of CP and Ca intake. In rats, a low protein diet resulted in significant reduction in intestinal Ca absorption and the development of hypo-calcuria (Gaffney-Stomberg *et al.*, 2010).

Shafey & McDonald (1991), however, reported no interaction between Ca and CP in broiler growth although a high dietary Ca level resulted in a depressed mineral utilisation and nitrogen digestibility thereby affecting feed conversion efficiency (FCE).

In humans, Ca plays a role in bone health, and there is a negative relationship between bone health and high CP intakes which shows that Ca is not adequate to protect the skeleton from high CP levels which may result in bone loss and skeletal problems (Heaney, 2002). Dawson-Hughes (2003) reported a positive impact of increasing CP intake on bone health of elderly people meeting

their dietary Ca requirement but not in elderly people who were not meeting their required intakes. Kerstetter *et al.* (2003) reported that at low Ca intakes in humans, high CP diets increased the absorption of Ca in the digestive tract resulting in an increase in urinary Ca which is associated with improved Ca balance. It was further reported that low CP diets reduced intestinal absorption of Ca. This agreed with a study later on where overall intestinal Ca absorption increased in response to a high CP diet (2.1 g/kg) when compared to a moderate CP diet (1.0 g/kg) (Kerstetter *et al.*, 2005).

Although there has not been any work found on the relationship between dietary CP and Ca in broiler breeders in particular, the studies done on mammal and other animal models may aid in understanding the correlation in broiler breeder males. The inconsistent findings on other studies suggest that there may be an effect of the level Ca on CP absorption and vice versa, and due to the lack of research may be important to investigate.

2.8 **Conclusions**

Broiler chickens grow rapidly and are fed ad libitum to attain the target weight at slaughter. Broiler breeders are different to their offspring because if fed ad libitum after about 4 weeks of age, BW will not adhere to the standard growth curve resulting in lifelong implications in the production phase such as a decrease in mating ability, fertility and eventually overall flock reproductive efficiency. The growth of broiler breeders is therefore limited through the use of restricted feeding seeing that birds fed ad libitum are associated with a number of risks.

For practical purposes, broiler breeder males may be fed a female ration but this practice has been challenged in recent years. Females require higher levels of CP and Ca mainly for egg production whilst males do not, and despite decreased feed allocations, the nutrient balance in the diet may not be adequate for male requirements. Due to these differences in requirements there is a need to investigate the possible implications of feeding a female ration on male fertility as the excess nutrients may have a negative effect. Published literature is inconclusive about the effects of CP and Ca on male fertility and hence further research is required to better understand how crude protein and calcium and their possible interaction affect fertility of male broiler breeders.

Therefore, the aim of this thesis is to determine the effect of different calcium and crude protein intakes on semen quality using the traditional and more recent measures of sperm quality and hence make a recommendation about the implications of feeding males a female diet on fertility

CHAPTER 3

THE EFFECTS OF DIETARY CRUDE PROTEIN AND CALCIUM ON FERTILITY OF MALE BROILER BREEDERS

3.1 **Introduction**

Feed management contributes a significant proportion of broiler breeder flock management and reproductive efficiency. Broiler breeders are primarily egg producers and growth needs to be limited to maintain fertility with age (Sahraei, 2012). Hatchability is the overall measure of both female and male fertility and while the number of hatching eggs produced also gives a good indication of female fertility, the assessment of male fertility is more complex. Assesment of spermatozoa quality has been used as an attempt to gauge male fertility. This includes sperm concentration (Hocking & Bernard, 1997b), volume (Brown & McCartney, 1983), mobility (Froman & McLean, 1996; Froman & Feltman, 1998) and sperm penetration of the IPVL of eggs (Bramwell *et al.*, 1995).

A proper feeding regimen to control BW in rear and production is essential. Once in production, the calcium and protein requirement of male broiler breeders is lower than that of female breeders. Separate-sex feeding during production has made it possible to allocate different rations to male and females but some breeder producers are still feeding males a female ration due to the impracticalities of small orders of feed for males and while feed allocation can be controlled, the diet formulation probably results in males consuming CP and Ca excess to requirements.

Various studies have investigated the effect of CP on fertility and sperm quality traits. Some have suggested that feeding levels between 12 and 14 % CP to broiler breeder males had no effect on sperm concentration, semen volume, number of sperm per ejaculate and fertility (Wilson *et al.*, 1987a; Wilson *et al.*, 1987b; Revington *et al.*, 1991) while others have shown that levels of 15 % and above significantly impair spermatozoa concentration (Hocking & Bernard, 1997b), number of males producing semen (Wilson *et al.*, 1987a), semen volume (Zhang *et al.*, 1999) and fertility (Hocking, 1990). Fontana *et al.* (1990), however, reported no significant differences in fertility of male breeders given either low (12%) or high (14%) levels of crude protein. Recently it has been reported that to maximise fertility and prolong the fertile period, broiler breeder males may be fed an optimum of 12.6% CP (Tyler & Bekker, 2012).

Calcium is an important nutrient for egg production in female broiler breeders and may be excess to the requirements in males even at lower feed allocations. Male breeders require lower Ca quantites, with reports suggesting that males require 0.2 g Ca/bird/day (NRC, 1994) and 0.90% Ca (Rosa *et al.*, 2010) during the production phase.

Oversupplying CP and Ca to males may be limiting the fertility of birds and is probably costly. Although the Ca in a female ration has not been reported to impair male broiler breeder fertility when fed female diets, a high CP was associated with a decline in fertility (Hocking, 1990). Due to the reported significant role of male fertility on overall flock fertility (Wolc *et al.*, 2009), obtaining the maximum male fertility could result in maximum number of hatching eggs without increasing flock size.

The possible effects of feeding a female ration to male broiler breeders and the interaction of the excess CP and Ca on male reproduction efficiency in the production stage remains uncertain. Therefore, the objective of this experiment was to study the effects of 4 different diets made up of 2 levels of CP and 2 levels of Ca (based on male and female requirements) on semen quality in the production phase using sperm concentration, mobility and spermatozoa penetration of the IPVL of oviposited eggs of male broiler collected days after insemination.

3.2 **Materials and methods**

3.2.1 **Experimental design**

Sixty Ross 308 broiler breeder males were obtained at 24 weeks of age from National Chicks Pty Ltd. Each bird was placed in an individual cage on the floor (60 cm X 35 cm X 40 cm) on arrival, which was equipped with shavings, a feed trough and a nipple drinker providing clean water provided *ad libitum*. The birds were fed a standard breeder male ration with feed intake adjusted to meet the breeder recommended growth curve until 32 weeks of age and during this time were trained for semen collection using the abdominal massage method (Burrows & Quinn, 1937). Forty eight males that had a consistent response were selected for experimental purposes at 32 WOA. There were six rooms considered blocks, each containing 8 cages. Individual sperm mobility values were measured and used to rank birds according to their mobility index value before allocating to treatments. Birds were then assigned to the six rooms with two males per room on each treatment until 61 WOA.

A 2X2 factorial design was used with 2 levels of CP (12.5 & 14.5%) and 2 levels of Ca (0.7 & 3.0%) which were formulated using Winfeed® (Table 3.1) to result in 4 treatment diets HP:LC,

LP:HC, HP:HC and LP:LC (Table 3.2). An ANOVA confirmed there were no significant differences in average body weight of males between rooms at the start of the experiment.

Table 3.1 *The ingredient content of the treatment diets differing in CP and Ca*

Ingredient	Unit	HP:LC	LP:HC	HP:HC	LP:LC
yellow maize fine	g/kg	698.13	741.83	657.25	717.76
wheat bran	g/kg	100.00	73.65	67.04	170.00
soybean 50	g/kg	74.78	89.00	166.36	
sunflower 37	g/kg	95.40			80.08
L-lysine HCl	g/kg	1.59	0.40	0.02	1.91
DL methionine	g/kg	1.30	0.88	1.59	0.69
vit+min premix	g/kg	1.50	2.50	1.50	2.50
limestone	g/kg	13.50	77.53	77.02	14.44
salt	g/kg	2.36	4.75	2.76	2.50
monocalcium phosphate	g/kg	8.39	9.47	9.56	7.08
sodium bicarbonate	g/kg	3.05		0.94	2.88
oil - sunflower	g/kg			15.97	

Table 3.2 *The nutrient content of the diets*

Nutrient (as is)		Treatment			
		HP:LC	**LP:HC**	**HP:HC**	**LP:LC**
Energy	MJ/kg	16.08	15.05	15.25	15.92
CP	%	14.46	12.41	14.65	11.86
NDF	%	16.92	10.56	11.61	17.69
ADF	%	6.70	4.07	4.91	6.50
Fat	%	3.31	2.98	3.56	3.08
Ash	%	5.35	9.71	11.06	4.78
Moisture	%	10.43	10.07	9.90	10.50
Ca	%	1.02	3.05	3.04	0.74
Mg	%	0.22	0.20	0.23	0.21
K	%	0.66	0.57	0.70	0.54
Na	%	0.20	0.23	0.16	0.20
P	%	0.63	0.52	0.53	0.59
K/ Ca +Mg	%	0.25	0.09	0.11	0.26
Zn	mg/kg	120.00	126.00	106.00	170.00
Cu	mg/kg	21.00	20.00	18.00	26.00
Mn	mg/kg	103.00	155.00	112.00	180.00
Fe	mg/kg	181.00	173.00	158.00	188.00

Nutrient content of the diets were determined using methods outlined by the Association of Official Analytical Chemists (AOAC) Official Method 990.03 (1995) for CP, McNab & Fisher (1984) for Energy AMEn (MJ/kg).

Birds received a feed intake of 140 g of feed per day, which was finished within an hour of feeding and therefore feed intake was not recorded. Body weight was measured on a monthly basis. Photoperiod was controlled with birds being provided 13 h light/day for the duration of the experiment. Sperm samples were collected every 3 weeks and analysed.

3.2.2 **Fertility Measurements**

3.2.2.1 **Sperm concentration**

Sperm concentration was determined using a Turkey Mobility Analyser 591B (Animal Reproduction Systems, Chino, CA) calibrated for use in chickens. A disposable pipette was used to draw 11.4 µl of neat semen which was then diluted with 3.42 ml of 3% (w:v) saline solution in a disposable cuvette. This was mixed and inserted into the specimen compartment of the Turkey Mobility Analyser which uses spectrophotometry to determine sperm concentration (Howarth, 1995).

3.2.2.2 **Sperm mobility**

Sperm mobility using the same sperm mobility analyser was measured on the same sample after determining sperm concentration. A sample of semen (50 µl) was diluted to contain 5 X 10^8 sperm/ml with sperm mobility buffer (Animal Reproduction Systems, CA) from the calculation of concentration and mixed. A pipette was then used to draw 300 µl of the buffered semen solution which was laid carefully on top of a 3% (w/v) Accudenz solution in a cuvette, incubated at 41^{o}C for 5 minutes and inserted in the mobility analyser, which read the absorbance at 550nm to return a mobility index from a precalibrated curve (Froman & McLean, 1996).

3.2.2.3 **Inner perivitelline layer sperm holes**

Semen was collected every 3 weeks from 6 individual males per treatment chosen from random rooms for the duration of the trial for the purposes of artificial insemination (AI) and measurement of sperm penetration days post-AI (separate to concentration and mobility analysis). Sperm concentration was measured as described above and sperm diluted with sperm motility buffer (Animal Reproduction Systems, CA) to contain 150 X 10^7 sperm/ml and the sperm used to inseminate a total of 72 commercial egg-type hybrids at 41 WOA (3 hens/male) using a 50 µl volume of the diluted sample for each hen, resulting in 5 million sperm being inseminated per hen. Although the same hens were used throughout and were therefore also aging, fertility of egg-type hybrids is good and unlikely to have affected the results.

Eggs were collected on days 2, 3, 4 and 8 post-insemination and stored in a cold room averaging 14^0C. All eggs from each treatment were analysed for the number of holes in the inner perivitelline layer (IPVL). Eggs were brought to room temperature, opened and the yolk separated from the albumen. Any excess albumen was then separated by rolling the yolk across a paper towel. An area of 1 X 1 cm of the perivitelline layer overlying the germinal disc was cut and immersed in a phosphate buffer solution to remove any adherent yolk. The resulting perivitelline layer was placed on a microscope slide, fixed with 4% formalin, stained with Schiff's reagent and covered with a cover slip and observed under a light microscope at 4X magnification (Bramwell *et al.*, 1995).

Images were captured using Motic Image plus 2.0 (Figure 3.1) and all unstained points of sperm penetration were quantified in a 2 x 2 mm square around the germinal disc using Image J 1.48, and the number of sperm holes per mm^2 were calculated.

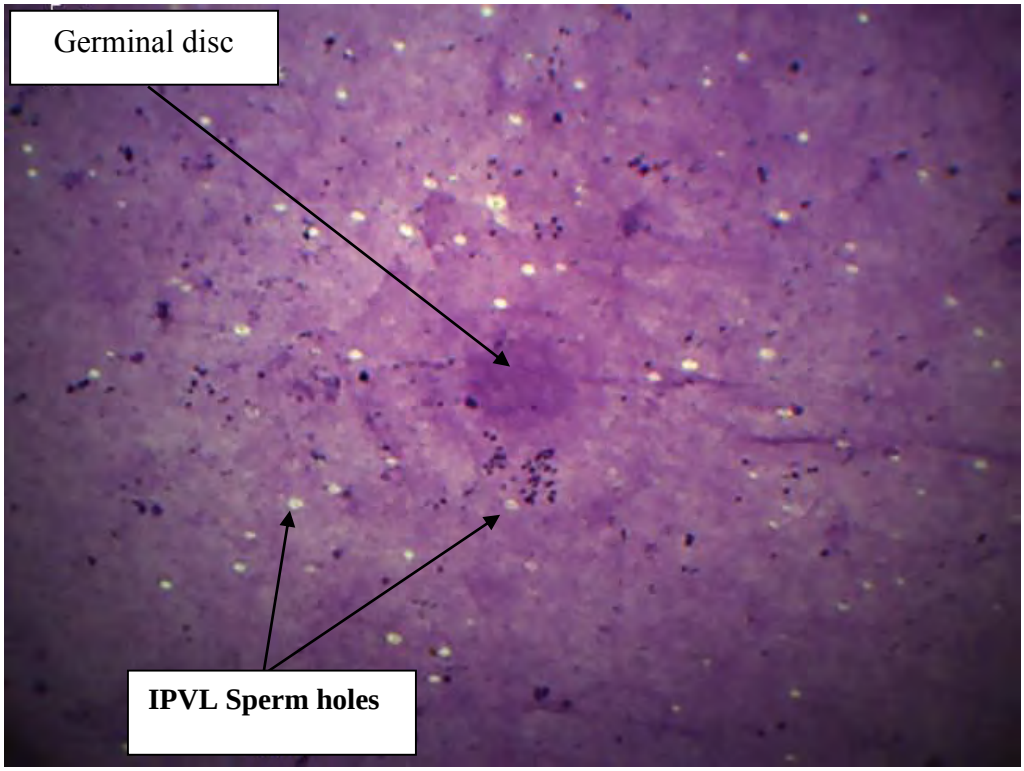

Figure 3.1 *Inner perivitelline layer sperm penetration holes around the germinal disc at 4X magnification.*

3.2.3 **Statistical analysis**

Unless stated otherwise, all collected data was statistically analysed using GenStat 14th Edition (VSN international Ltd., 2013).

Body weight data were subjected to a general ANOVA and analysed at each age. Treatment means were compared using LSD values at the 5% significance level.

The sperm concentration and sperm mobility data were subjected to an unbalanced general ANOVA design due to the different number of male birds from each treatment that produced semen on the days of measurement. The data were analysed for the overall period and at each age. Treatment means were compared using LSD values at the 5% level of significance.

Data were tested for normality using the Shapiro-Wilks test and that of IPVL sperm holes was observed not to be normally distributed. The data were therefore log transformed by adding a constant (to avoid a log of 0) and performing a log transformation. The log number of IPVL sperm penetration holes observed from the oviposited eggs on days 2, 3, 4, and 8 post-AI were subjected to a general ANOVA using an unbalanced treatment structure because some eggs were lost during data collection resulting in an unequal number of eggs for each treatment. The mean log IPVL sperm holes at each day post-AI were compared using LSD values at the 5% level of significance. Although the ANOVA and mean comparisons were performed on log-transformed data, the result presented in Table 3.7 is the actual data.

3.3 **Results and discussion**

3.3.1 **Body weigh**t

There were no significant differences observed in mean BW at 32 WOA, however differences in BW were observed from 37 WOA (after birds were fed different treatments for 5 weeks) to the end of the trial at 61 WOA, where birds with high CP and high Ca intake had significantly higher ($p<0.05$) BW (Table 3.4). There was no difference in BW of the other treatments. This was unexpected because high dietary CP was expected to result in heavier male broiler breeders, but this group were similar to those fed low CP.

Romero-Sanchez *et al.* (2008) and Zhang *et al.* (1999) reported that higher CP intake resulted in higher BW of male broiler breeders. This was the case in birds fed HP:HC but not those fed HP:LC, which indicates possible problems with the feed allocation. Wilson *et al.* (1987b) who reported a significant BW loss from males that were fed low CP level (10.8 g CP/bird/day) while Wilson *et al.* (1987a) observed no significant change in the BW of male broiler breeders with differing CP intakes.

Table 3.4 *Body weight of broiler breeder males with different CP and Ca intakes*

Age (wks)	Treatment	BW (kg)	Lsd	p-value
32	HP: LC	4.496	0.1900	0.325
	LP: HC	4.442		
	HP: HC	4.388		
	LP: LC	4.557		
37	HP: LC	4.879[ab]	0.1981	0.125
	LP: HC	4.683[a]		
	HP: HC	4.900[b]		
	LP: LC	4.790[ab]		
41	HP: LC	4.973[ab]	0.2149	<0.001
	LP: HC	4.765[a]		
	HP: HC	5.179[b]		
	LP: LC	4.773[a]		
47	HP: LC	5.005[a]	0.2856	<0.001
	LP: HC	4.829[a]		
	HP: HC	5.338[b]		
	LP: LC	4.829[a]		
52	HP: LC	5.142[a]	0.2744	<0.001
	LP: HC	5.031[a]		
	HP: HC	5.698[b]		
	LP: LC	4.948[a]		
57	HP: LC	5.175[a]	0.3306	<0.001
	LP: HC	5.142[a]		
	HP: HC	5.826[b]		
	LP: LC	5.053[a]		
61	HP: LC	5.313[a]	0.3728	<0.001
	LP: HC	5.188[a]		
	HP: HC	6.094[b]		
	LP: LC	5.173[a]		

[a, b] – means with superscripts that differ are significantly different (P<0.05)

3.3.2 **Sperm concentration and mobility**

Crude protein intake had no significant effect on sperm concentration at each age (Table 3.5). There was a significant main effect of Ca on sperm concentration at 42 WOA. Male broiler breeders fed a HP: HC diet had significantly lower sperm concentration at 42 WOA and 57 WOA than males subjected to a HP: LC diet. A Significant interaction ($p<0.05$) between CP and Ca intake was observed at 60 WOA, where male broiler breeders fed on a HP: HC diet had significantly lower sperm concentration than males fed HP: LC.

A number of studies observed no significant impact on sperm concentration when breeder males were fed diets differing in CP (Wilson *et al.*, 1987a; Wilson *et al.*, 1988; Revington *et al.*, 1991; Zhang *et al.*, 1999). However, Hocking & Bernard (1997b) observed a significantly higher concentration of sperm in broiler breeder males fed on a high CP diet (160 g CP/kg) than those on a low CP diet (120 g CP/kg). Although not significant, Hocking (1989) observed a negative relationship between dietary CP level and sperm concentration concluding that any effects of CP on fertility are not possibly linked to semen yield and quality but rather to male mating behaviour. Calcium intake does not seem to significantly impair nor improve sperm concentration of male broiler breeders at any age although numerically it was observed that males fed on a HC diet had lower concentration values.

Crude protein and Ca intake had no significant effect on sperm mobility at each age (Table 3.6). Although there has not been work done on the effect of diet alteration on sperm mobility, the fertility of broiler breeder males is understood to be a function of the sperm mobility trait

(Donoghue *et al.*, 1999**;** Froman *et al.*, 1999), therefore regarded to be a more reliable parameter than sperm concentration.

Table 3.5 *Effects of Crude protein (CP) and Calcium (Ca) intake on mean sperm concentration from 36 to 60 WOA*

	Sperm concentration (sperm/ml (x10^6))													
Trt	**36**	**n**	**39**	**N**	**42**	**n**	**45**	**n**	**49**	**n**	**53**	**n**	**57**	**n**	**60**	**n**
HP:LC	7.96	11	7.96	12	8.30[a]	12	8.73	11	9.26	11	9.46	11	10.35[a]	11	11.33[a]	11
LP:HC	7.40	12	8.13	12	7.50[ab]	12	8.65	9	8.67	11	9.66	11	8.96[ab]	10	10.72[ab]	10
HP:HC	7.44	11	6.99	11	6.72[b]	12	8.53	5	7.46	12	8.25	11	8.34[b]	11	8.93[b]	8
LP:LC	8.30	12	7.71	12	8.22[a]	12	8.35	11	9.03	12	10.11	12	9.28[ab]	11	10.26[ab]	11
p																
CP	0.78		0.33		0.45		0.99		0.37		0.16		0.55		0.71	
Ca	0.16		0.55		0.02		0.97		0.12		0.28		0.07		0.36	
CP*Ca	0.64		0.11		0.35		0.59		0.31		0.62		0.23		0.04	
sed																
Min	0.66		0.6				0.85		0.89		0.94		0.77		0.9	
average	0.67		0.61		0.65		1		0.92		0.95		0.8		0.95	
Max	0.69		0.61				1.15		0.94		0.96		0.83		0.99	
Lsd																
Min	1.33		1.21				1.75		1.81		1.91		1.56		1.83	
average	1.36		1.22		1.31		2.05		1.85		1.93		1.62		1.93	
Max	1.4		1.24				2.36		1.9		1.95		1.69		2.03	

[a, b] – column means with superscripts that differ are significantly different (P<0.05)

Table 3.6 *Effects of Crude protein (CP) and Calcium (Ca) intake on mean sperm mobility % from 36 to 60 WOA*

	Sperm mobility %															
Trt	**36**	**N**	**39**	**N**	**42**	**n**	**45**	**n**	**49**	**n**	**53**	**n**	**57**	**n**	**60**	**n**
HP:LC	16.64	11	15.50	12	11.75	12	14.64	11	15.73	11	21.09	11	18.82	11	13.64	11
LP:HC	11.17	12	10.33	12	11.67	12	11.56	9	14.36	11	18.36	11	21.10	10	13.70	10
HP:HC	11.82	11	11.82	11	18.42	12	11.60	5	17.25	12	20.45	11	25.80	11	13.88	8
LP:LC	13.00	12	10.17	12	12.00	12	11.27	11	15.58	12	18.83	12	21.17	11	13.18	11
p																
CP	0.26		0.19		0.34		0.38		0.4		0.36		0.79		0.99	
Ca	0.12		0.60		0.35		0.47		0.99		0.71		0.23		0.96	
CP*Ca	0.46		0.55		0.31		0.19		0.50		0.86		0.26		0.96	
sed																
min	2.9		3.91				2.27		2.64		3.12		3.75		3.71	
average	2.97		3.96		4.78		2.67		2.71		3.16		3.71		3.94	
max	3.05		4.01				3.06		2.78		3.2		4.07		4.13	
Lsd																
min	5.87		7.91				4.65		5.36		6.32		7.62		7.58	
average	6.02		8.01		9.67		5.47		5.49		6.40		7.94		8.03	
max	6.17		8.11				6.28		5.63		6.48		8.26		8.42	

– column means with no superscripts are not significantly different (P>0.05)

3.3.3 **IPVL Sperm holes**

There were no significant differences found in the log number of IPVL sperm holes of eggs inseminated with sperm from males fed varying CP and Ca levels at any age ($p>0.05$). There was no interaction between CP and Ca on the number of sperm holes found in the IPVL of eggs. There was an expected significant effect of day PAI on the log number of IPVL sperm holes in males on each treatment, where the number of sperm holes in the IPVL of oviposited eggs decreased with increasing day PAI (Table 3.7). At each age, the average IPVL sperm holes on days 2 and 3 PAI were consistently higher (>6) while IPVL sperm holes on day 8 PAI was lower than six, which could mean that the eggs were no longer fertile at day 8 PAI (Wilshart, 1997).

The probability for fertilisation to occur decreases with increasing days PAI (Wishart, 1987; Bramwell *et al.*, 1996**;** Fasenko *et al.*, 2009), because the SST are filled by fecund spermatozoa after mating or insemination (Bakst *et al.*, 1994) and sequentially released to the infundibulum providing fertile eggs for a limited period (Brillard, 1993). As in this study, Bramwell *et al.* (1996) observed a decline in mean flock sperm holes in the IPVL of oviposited eggs with age. Similarly, Hazary *et al.* (2000) also looked at the IPVL points of hydrolysis (sperm holes) on oviposited eggs between 30 to 55 WOA and observed that the total number of sperm holes declined from 200 at peak fertility to less than 20 at 55 WOA whilst mean proportion fertile eggs decreased by 15% between peak fertility and 55 WOA. The decline in IPVL sperm holes with age may not be related much to nutrient alteration of the diet fed to the males but rather related to the natural age-related fertility decline observed in male broiler breeders late in the production period (Hazary *et al.*, 2000; Hocking & Bernard, 2000), and it is possible that this was exacerbated by increasing age of the egg-type hybrid females used to assess IPVL holes.

It has however been reported that the fertilising and sperm penetrating abilities of sperm from males is not affected by age (Bramwell *et al.*, 1996), suggesting that mating frequency may be an important factor for successful sperm: egg interaction. The filling of the SST is essential for successful IPVL sperm penetration and hence fertilisation following an insemination or mating (Bakst *et al.*, 1994; McGary *et al.*, 2002). Therefore, a high mating frequency needs to be prioritised to ensure that the SST are filled with sperm and thus fertility is maintained throughout the production period and the age-related decline in fertility is minimised.

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Table 3.7 *Mean IPVL sperm holes with days post-AI at each age* (The ANOVA and mean comparisons for IPVL sperm holes were performed on log-transformed data, but shown are actual sperm holes)

		Treatment			
Age (WOA)	**DPAI**	**HP:LC**	**LP:HC**	**HP:HC**	**LP:LC**
37	2	11.2	16.1	12.6	11.7
	3	7.3[a]	9.4[b]	10.9[b]	7.8[a]
	4	7.0	8.0	8.7	6.8
	8	3.2[b]	1.8[a]	2.8[ab]	2.4[ab]
40	2	8.2	12.6	8.7	8.7
	3	8.8[a]	8.7[a]	8.8[a]	14.3[b]
	4	7.0[a]	7.2[a]	5.6[a]	9.8[b]
	8	2.6[a]	2.5[a]	2.5[a]	3.8[b]
55	2	6.7[a]	6.9[a]	16[c]	9.4[b]
	3	6.3	5.4	8.3	8.3
	4	3.0	3.4	3.1	4.9
	8	2.9	1.7	2.8	2.9
59	2	3.7	5.4	6.8	4.8
	3	7.0	6.9	3.5	8.8
	4	3.3[ab]	2.8[a]	2.1[a]	4.8[b]
	8	2.1	1.3	1.8	2.0

[a - c] – Row means with superscripts that differ are significantly different ($P<0.05$)

3.4 **Conclusion**

Different CP and Ca intakes of broiler breeder males during the production phase (32- 61 WOA) had a significant effect on BW. As expected, feeding a female ration (HP: HC) to male broiler breeders resulted in heavier males compared to feeding a separate male ration (LP: LC) and this could have an adverse effect on male mating efficiency hence it may be more beneficial to feed a low nutrient content diet as it allows for better control of BW. It was however unexpected that the

BW of males fed HP: LC would be similar to that of males fed the low protein rations, therefore this may show that feeding either of the diets does not significantly impair nor improve male broiler breeder BW.

Dietary intake of CP had no significant influence on sperm mobility while it was observed to significantly influence sperm concentration at certain ages but use of sperm concentration as a sperm quality measure has been thought to be unreliable compared to mobility and sperm penetration. Calcium intake had no significant influence on sperm mobility at all ages but high Ca intake significantly decreased sperm concentration at 42 WOA. The effect of Ca on fertility parameters in male broiler breeders has not been assessed before but work in human beings and broilers is not supported by these results as Ca did not significantly impair semen quality traits nor have an interaction with CP whilst a number of authors have reported no significant effect of CP intake on spermatozoa concentration. There is limited work on CP effect on sperm mobility but in this study, the CP and Ca intakes used resulted in no significant effect on sperm mobility at each age.

There were no significant differences in the number of IPVL sperm penetration holes from males fed diets differing in CP and Ca and it can be concluded that dietary intake of CP and Ca at the levels used in this experiment does not seem to improve nor impair the fertility of male broiler breeders.

Therefore, it can be concluded from these results that male broiler breeders could be fed a specific male ration lower in protein and calcium (12.5 % and 0.7 %) than female specifications. This enables better control of male development and growth, and has the potential to maintain fertility while saving the farmer an unnecessary protein cost. It is also evident from this study that feeding

male broiler breeders a female ration (14.5 % CP and 3.0 % Ca) does not result in the males becoming less fertile than males fed a specific male ration (12.5 % CP and 0.7 % Ca) and it can therefore be concluded that it does not matter if broiler breeder males are fed on a female ration, rather it would be cheaper to feed broiler breeder males a separate male ration consisting of low nutrients but the impracticalities of ordering small feed quantities and having to prepare separate storage facility cannot be ignored.

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