THE EFFECT OF CRUDE PROTEIN INTAKE ON FERTILITY IN YOUNG AND OLD MALE BROILER BREEDERS

by

BRENDAN GRAAF
BSc. Agric., University of KwaZulu-Natal, Pietermaritzburg

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University of KwaZulu-Natal
Pietermaritzburg

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ABSTRACT

Due to genetic selection and improvements in broiler growth traits there have been negative influences on fertility in broiler breeder parents. This is mainly related to excess body weight gain resulting in the inability to achieve successful cloacal contact during copulation and problems with the hierarchical formation of follicles in the ovary. Male broiler breeders are often fed a female ration which contains crude protein (CP) requirements for egg production, and may not be necessary for males. Protein is one of the most costly components of poultry feed, and overfeeding protein has a number of downsides. The impact of sub-fertile and unfertile males in the overall fertility of the flock could be large. Maximizing male fertility could ensure maximizing fertile egg production which would result in more broiler chicks without increasing the size of the breeding flock.

The objective of this experiment was to investigate the effects of three different dietary CP intakes (96.8, 117 and 130 g CP/kg, low, medium and high respectively) on fertility in young and old male broiler breeders using some old and more recent fertility measures in an attempt to determine whether it would be justified to feed males a separate ration. Results showed that across all male ages no significant response in body weight (BW), sperm concentration and sperm mobility to dietary CP intake were seen. Although in the young males, birds on the high CP intake showed significantly ($P<0.05$) lower mean BWs than males on the low and medium CP intakes, and sperm mobility values were seen to be highest in birds receiving the medium CP intake across all male ages. The log number of inner perivitelline layer (IPVL) sperm holes was seen to increase with increasing CP intake ($P<0.001$) in males between 42 and 62 weeks of age (WOA) but showed no response in males from 27-41 WOA. The log number of IPVL sperm holes was seen to generally decrease with age in males from 27-60 WOA; however eggs collected two days post-artificial insemination (PAI) had a similar log number of IPVL sperm holes, regardless of treatment, throughout the study. The mean number of IPVL sperm holes was seen to decrease as days PAI increased. There was a tendency for a superior response in fertility, predicted from IPVL sperm holes, from birds on the medium protein intake.

PREFACE AND DECLARATIONS

The experimental work described in this dissertation was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, from February 2011 to December 2013, under the supervision of Dr Nicola C. Tyler and Dr M Ciacciariello.

I, ……. Declare that

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

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ABBREVIATIONS USED IN THE TEXT

- µl - micro litre
- AI - artificial insemination
- ANOVA - analysis of variance
- BW(s) - body weight(s)
- CP - crude protein
- IPVL - inner perivitelline layer
- LSD - least significant difference
- ME - metabolisable energy
- MJ - mega joules
- OPVL - outer perivitelline layer
- P - probability
- PAI - post artificial insemination
- R - rand
- s.e.m. - standard error of mean

CHAPTER 1

GENERAL INTRODUCTION

With the use of intense genetic selection as well as improved nutritional management, the broiler industry has successfully improved the feed conversion rate, breast muscle yield and body weight (BW) of the modern broiler, as well as decreasing the age to slaughter (Havenstein *et al*., 2003; Romero-Sanchez *et al*., 2007a,b). However the same intense selection for growth rate and feed efficiency in broilers has been detrimental to the parent stock (broiler breeders). Under conditions of *ad libitum* feeding broiler breeders will consume feed at near gut capacity (Nir *et al*., 1978) resulting in higher BWs, obesity, increased carcass fat and a higher incidence of foot and leg disorders in males (Hocking & Duff, 1989; Emmerson, 1997). This has led to a reduction in reproductive performance, such as delayed sexual maturity, reduced ability to mount and therefore copulate successfully and reduced fertility (Hocking & Duff, 1989; McGary *et al*., 2002; Romero-Sanchez *et al*., 2007a,b).

In order to maintain high flock fertility and control BWs of the birds in commercial conditions the broiler breeder industry is forced to employ a number of management strategies such as feeding a quantitatively restricted ration during the growing period in order to limit BW at sexual maturity (Hocking *et al*., 1989) as well as structured feed restriction programs during production. Selection for fertility traits and photoperiod management is also necessary in this regard.

The nutrition of male broiler breeders is often overlooked, and males are often fed a female diet, only with a greater degree of feed restriction. The crude protein (CP) requirement of

male and female broiler breeders differs as the protein requirement of the birds is dependent on the reproductive processes which utilize amino acids (Fisher, 1998). The female CP requirement in the production phase is generally higher than that of the male due to the higher requirement for egg production compared to spermatogenesis. Overfeeding, or incorrect feeding, of CP can have a number of downsides such as the energy cost associated with excreting excess nitrogen from the body (Hocking, 1989) as well as the high heat increment from metabolism (Coon, 2004). Crude protein is also one of the most costly components in poultry feed and therefore it is essential to determine the optimum levels of CP required for maximum male fertility.

The literature concerning CP requirements and fertility in broiler breeder males is limited and the findings inconsistent, probably due to the variation in techniques used to assess male fertility, as well as the effectiveness of such techniques in predicting male fertility.

The aim of this experiment was to use some older techniques as well as some more recent techniques in predicting male broiler breeder fertility to determine the optimum intake of CP using a range of CP intakes (12.6-18.9g CP/bird/day) in young and old males, and also to assess the effects of cumulative CP intake on fertility.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Broiler growth rates have been shown to be negatively correlated to fertility traits (Hocking & Duff, 1989; Lake, 1989; Nir *et al*., 1996; McGary *et al*., 2002) and broiler breeder flock fertility during the early breeding period (30-49 WOA) is reported to be above 95 percent, but declines rapidly during the latter weeks of production (Walsh & Brake, 1997; Casanovas, 2002). Therefore, proper feeding and management of broiler parent stock is key to maintaining flock fertility and ensuring maximum numbers of fertile eggs during the production cycle.

The following review will cover the literature concerning the reproductive physiology of the broiler breeder male, the nutritional requirements (energy and protein) and their effect on male fertility. This will be reviewed during the rearing phase by focusing on how nutrition and management affect subsequent sexual maturity and fertility, while also addressing feeding practices implemented to sustain fertility during the production phase. Lastly some old and more recent measures of semen quality and their accuracy in predicting male fertility are discussed. Intakes (crude protein and metabolisable energy) were calculated where possible, but reported as percentages or as g CP/kg where it was not possible to calculate due to feed intakes not being reported. This review covers work published some time ago, and where possible, is linked to more recent work.

2.2 MALE REPRODUCTIVE PHYSIOLOGY

2.2.1 Gonads

The function of the male is to produce viable sperm in the testes and in the case of natural mating, to copulate and efficiently transport these sperm cells to the female reproductive tract via the cloaca (Leeson & Summers, 2000). The male has two functional testes, unlike in the female where only the left ovary is functional, since the right ovary degenerates prior to hatching (Jull, 1940; Etches, 1996). The paired testes of the rooster, unlike most mammals, are situated within the body cavity and thus function at normal body temperature of close to 41°C (Lake & El Jack, 1966; Leeson & Summers, 2000). The testes are small yellowish-coloured bodies situated at the anterior ends of the kidneys attached by ligaments to the dorsal body wall on either side of the midline (Lake & Stewart, 1978; Leeson & Summers, 2000). Male domestic fowl, unlike mammals do not possess certain accessory reproductive organs such as a prostate gland, seminal vesicles, Cowper's glands or glands of Littré; they also have an extremely short epididymis (Lake & El Jack, 1966).

Prior to maturity the testes are quite small, being only 1-2 g each, however, similar to the situation with the ovary in the hen, at around 18 weeks of age there is a dramatic increase in size, making the mature testes easily visible at around 15-20 g (Leeson & Summers, 2000). Although puberty in the male has been defined as the age of first appearance of spermatozoa in the ejaculated semen (between 16-24 weeks of age) the semen quality is initially poor, and so sexually maturity has also been defined as the time when testes growth as well as the number and quality of spermatozoa is maximal (de Reviers & Williams, 1984). A sexually active rooster will produce approximately three billion sperm daily, or about 100 million per gram of testes weight (Etches, 1996; Leeson & Summers, 2000). This daily production remains fairly constant and is unaffected by mating or collection frequency (Leeson & Summers, 2000). However overall peak sperm production

in roosters is seen at around 24 weeks of age and slowly diminishes with age (Van Krey, 1990).

2.2.2 Spermatogenesis

The spermatozoa are produced in the seminiferous tubules of the testes through the hormone regulated process of spermatogenesis (Etches, 1996). The spermatozoa are released into the lumen of the seminiferous tubules and swept towards the rete testis. The rete testis is lined with ciliated cells as well as contractile cells which aid in the transport of the spermatozoa to the epididymis. The rete testis and the epididymis also contain cells which synthesize and secrete proteins. It has been speculated that these proteins are related to the attainment of motility and fertilizing capacity of the spermatozoa. This is further backed by the observation that alteration or removal of proteins from the semen through the use of concentrated salts or exposure to neuraminidase prevented sperm transport (Etches, 1996).

The semen is then moved from the epididymis to the cloaca via the vas deferens, which also functions as a reservoir for semen prior to ejaculation. The transport of semen through the epididymis and the vas deferens is assisted by peristalsis of the muscular layers surrounding these ducts. It takes around 24 hours for sperm to reach the vas deferens from the epididymis. The volume of semen in the lower vas deferens after ejaculation is slightly larger than the previous ejaculate, suggesting that the reserve is slightly more than one or two ejaculations (Etches, 1996). If however after a 2-3 day period, ejaculation does not occur, then any sperm stored in the vas deferens are reabsorbed (Leeson & Summers, 2000). At ejaculation semen is expelled through papillae which extend the vas deferens into the urodeum. The ejaculate then flows into the proctodeum between the lateral phallic folds. Each phallic fold is engorged by lymph from the network of sinuses connected to the lymphatic system. The seminal fluid present in avian semen is derived from secretions of the entire lining epithelium of the genital tract. However the transparent fluid, also known

as accessory fluid, is added to the semen from lymph folds lining the cloaca (Lake & Wishart 1984; Van Krey, 1990; Etches, 1996). In some cases particularly in roosters, semen can be expelled from the vas deferens with such a force that it never touches the cloaca. Semen that does not come into contact with the cloaca is preferred for artificial insemination (AI) and *in vitro* manipulation of semen as it contains fewer contaminants from the gastrointestinal tract, ureters, and less lymph from the lymph folds of the cloaca (Etches, 1996).

In general the volume of an avian ejaculate when compared to a mammal is relatively small while the spermatozoa concentration is relatively large. The volume and concentration of the semen is largely influenced by the cloacal transparent fluid. Cloacal transparent fluids dilute semen by approximately 50% in domestic fowl (Van Krey, 1990).

2.3 NUTRITION AND FERTILITY

2.3.1 Nutrition in Rearing

The broiler industry has successfully used genetic selection to improve the expression of highly heritable traits such as feed efficiency, BW and breast muscle yield. This has generally implied a need to constantly adapt the management and feeding program of the broiler parent stock in order to avoid the potential decline in reproductive performance, such as delayed sexual maturity and reduced fertility associated with overweight birds (Romero-Sanchez *et al*., 2007a,b). Low density diets, CP level and feed restriction before and after sexual maturity are used to control BW of broiler breeder males in order to achieve optimum reproductive performance (Zhang *et al*., 1999; Leeson & Summers, 2000). However there is variation among commercial breeder producers in the level of CP in the diet, degree of feed restriction, and age at commencement of restriction (Zhang et al., 1999).

It has been shown that both female and male broiler breeders require a minimum cumulative nutrient intake of CP and ME during the rearing period in order to sustain subsequent egg production, fertility and reproductive performance during the production period (Walsh & Brake, 1997, 1999; Romero-Sanchez *et al*., 2004). It has been shown in male broiler breeders that differences in caloric intake during rearing affected both body composition and growth rate more than differences in dietary CP intake (Vaughters *et al*., 1987). Sexton *et al*, (1989a,b) and Cerolini *et al*, (1995) reported that lower ME intakes during the production period were responsible for decreased fertility and alterations in semen characteristics. Vaughter *et al*, (1987) showed that low CP diets fed to males during the rearing period had no effect on semen volume and spermatozoa concentration in production. Zhang *et al*, (1999) also showed that spermatozoa concentration, volume, number of spermatozoa per ejaculate and testes weight tested at 50 WOA were unaffected by CP levels (9, 12, and 15%) in the diet (isocaloric intake) before sexual maturity. Hocking *et al*. (2002) showed that females fed low protein rations during rearing were associated with higher rates of feed intake, lower rates of egg production and higher mortalities.

One of the major problems encountered with commercial breeder flocks is a decline in fertility during the latter part of the production period, particularly after 50 WOA (Walsh & Brake, 1997; Casanovas, 2002). This decline in fertility is caused by a progressive reduction in mating frequency, mating efficiency, or both, and has been largely attributed to the excessive BW and poor physical condition of older males (Hocking, 1990). However, there is some evidence that a deficient daily ME intake in the latter part of the production period could intensify the decline in late fertility (Sexton *et al*., 1989a,b; Cerolini *et al*., 1995; Bramwell *et al*., 1996a). Romero-Sanchez *et al*, (2007a,c) showed that the decline in late fertility could be restored by increasing the male feed allocation during the latter part of the production period, and clearly demonstrated that for males the quantity of feed during the production period played a more important role in late fertility than the feed allocation program during the rearing period. However, they do require a certain nutrient intake during the rearing period in order to achieve a required BW at

sexual maturity. However, if the males are too heavy at maturity it can lead to excessive BW later in production, which will also negatively affect their fertility.

2.3.2 Nutrition in Production

Broiler breeders have a nutrient requirement (metabolisable energy (ME), crude protein (CP), amino acids and minerals) for maintenance, growth and reproduction. During the early rearing phase (0-4 WOA) a 19% CP diet is recommended for both male and female broiler breeders, after which a 15-16% CP diet is recommended until 25 WOA (Arbor-Acres-Plus-Avigen, 2007). The CP requirement then decreases with age and levels off during the production stage, with female birds requiring a CP percentage between 14.5 and 15.5% and males between 12 and 14%. The protein requirement of the birds is dependent on the productive processes which utilize amino acids (Fisher, 1998). The female requirements are however generally higher than those of the male in the production phase, due to the nutrient requirements of egg production which is greater than that of spermatogenesis. Although energy and CP are the main focus for nutrient requirements, other nutrients such as vitamins, minerals and other specific amino acids are also vital for the general productivity and well-being of the birds (Barber *et al*., 2005).

Although CP and ME intakes will be looked at more closely, feed intake has an effect on male fertility as a lower or higher feed intake results in an under or over supply of required nutrients. Brown & McCartney (1983) determined the effects of feed intake on male broiler breeder fertility, semen volume, testes size, hatchability and BW. As feed intake decreased BWs were seen to significantly decrease. No significant effect was seen on fertility or hatchability. Testes size was seen to be greatest in birds fed 154 g/bird/day; however semen volume was greatest in birds fed 130.9 g/bird/day.

2.3.2.1 Crude Protein and its Effects on Male Broiler Breeder Fertility

Feed restriction programs predominantly control body weights (BWs) of the birds by varying the amount of feed consumed. In order to achieve different levels of BW gain, Hocking, (1990) fed naturally-mated broiler breeder males a high (16.1%) or low (11.3%) protein diet, and found that during the production stage suboptimum fertility resulted if BW was too much above or below the target. Males with excessively high BWs were incapable of copulating, whereas males with low BWs had compromised testes development and semen production. Fontana *et al*. (1990) observed significantly higher egg fertility when males were fed a lower CP diet than when they were fed the female ration. Semen concentration was found to be the same in males fed separately than those fed the same diet as the females. Thus, the increased egg fertility was attributed to the lower weight and size of the males and therefore their ability to copulate more successfully. This was the case 20 years ago; but due to the lack of current literature there is a need to investigate if this is still the case with improved genotypes.

Hocking & Bernard (1997a) determined the effects of CP and BW on overall fertility in two strains of broiler breeder males. Birds were fed a diet containing 12 or 16% CP. One of the strains receiving 16% CP was found to have lower fertility (measured as fertile eggs candled at 18 days of incubation per total egg set, as well as sperm trapped in the OPVL of egg) than the other, which was associated with higher breast muscle weight, a higher number of incomplete matings, and less frequent copulations. Hocking & Bernard (1997b) in a similar experiment showed that the sperm concentration was lower in males fed a diet containing 16% compared to 12% CP. It was also seen that males on the high protein diet had a smaller average testes size and spermatogenesis in the testes (measured by staining sections of testes with haematoxylin and eosin and scoring for the presence of spermatozoa) was absent in a large proportion of the males.

Borges *et al*. (2006a) observed a quadratic effect of CP intake on semen volume, sperm concentration, motility, spermatozoa vigour and fertility when broiler breeder males were fed one of five feed treatments (12.0, 14.2, 16.4, 18.6 and 20.8 g CP/bird/day). Birds on

extreme levels (deficient or excess) of CP intake showed increased carcass crude fat percentage and decreased reproductive performance. Body weights of the males were however not affected by the CP treatments. An intake of 16.9 g of CP/bird/day was recommended to meet the CP requirements and optimize fertility of broiler breeder males from 27-61 WOA. Further effects of CP on fertility will be discussed under 2.4.

2.3.2.2 Energy and its Effects on Male Broiler Breeder Fertility

Extensive research has been conducted in order to determine the optimum ME content in a diet; however findings among researchers have been inconsistent. Experiments on birds at different stages of production, measurements of different parameters (male fertility measurements, body weights) and reporting ME contents of the diets without feed intakes, all make it difficult to compare results.

Sexton *et al.* (1989a) fed individually-caged broiler breeder males one of five feed treatments differing in ME content (6.7, 8.4, 10.0, 11.7, and 13.4 MJ ME/kg feed) with a constant CP level (10%). Semen concentration, total spermatozoa per ejaculate, semen weight and percentage of males producing semen were shown to be linearly and positively related to energy level. Body weight, carcass fat percentage and protein percentage were shown to be quadratically related to dietary energy.

Attia *et al*. (1995) reported an increase in BW and testes weight with increasing energy intake during production in broiler breeder males. No significant effect was seen on fertility, semen characteristics or hatchability with increasing energy intake and carcass composition was found to be unaltered. It was also seen that the birds were able to maintain BW when fed diets low in ME. There was however concern that the spermatozoa maturation process may have been altered, affecting sperm motility and resulting in less spermatozoa reaching the sperm storage tubules, although there was no significant effect on fertility.

Borges *et al*. (2006b) observed that sperm motility, vigour and fertility were affected quadratically (starting low, increasing and then decreasing) by the level of ME intake in

male broiler breeders fed one of five feed treatments (1.21, 1.30, 1.38, 1.47 and 1.55 MJ ME/bird/day). It was also seen that BW as well as carcass fat and protein contents increased linearly as ME levels increased. Borges *et al*. (2006b) recommended an average level of 1.45 MJ ME/bird/day to meet the energy requirements of breeding males from 26-61 WOA.

2.3.3 Feed Restriction programs

It has been widely recognized that excessive BW gain will have adverse effects on the reproductive performance of broiler parent stock (Hocking & Duff, 1989; Nir *et al*., 1996; McGary *et al*., 2003); thus the use of feeding programs has become a standard management practice. There are a number of feeding programs which can be used to limit growth and improve fertility in broiler breeders. Some of these include limiting the quantity of feed given daily (quantitative feed restriction), alterations in the nutrient density of the feed (qualitative feed restriction) and using sex-separate feeding systems.

2.3.3.1 Quantitative Feed Restriction

Feed restriction programs vary considerably within the poultry industry with respect to the age at the onset of restriction, diet specifications, and the type of restriction used (Yaissle & Lilburn, 1998). Feed restriction is generally initiated when birds are one to three weeks of age, with feed intakes during rearing and production being 60-80 and 25-50 percent less, respectively, than birds would consume *ad libitum* (Mench, 2002). In adult birds this results in a reduced BW of approximately 45-50 percent than that of *ad libitum* fed birds (Katanbraf *et al*., 1989).

There are two commonly used commercial feed restriction programs, the skip-a-day (SAD) and limited-every-day (LED) feeding program (Mench, 2002). The SAD program uses amounts of a balanced feed ration calculated to achieve desired BWs fed on alternative days. However due to animal welfare reasons, this practice has been banned in the United

Kingdom and many other countries in Europe (Hocking, 2004). The preferred program is the LED, where a limited amount of balanced feed is fed on a daily basis (Mench, 2002).

Brown & McCartney, (1983) found that restricted feeding in males had no significant effects on the fertility or hatchability of eggs produced by females that had been artificially inseminated, indicating that reduced fertility in natural-mating flocks is most likely due to changes in body conformation. As expected, BW gains were significantly lower as the degree of restriction was increased. In a similar study Brown & McCartney, (1986) fed individually caged male broiler breeders varying levels of restricted feed (115, 100, 85, 70 and 55 percent of the required intake) in order to determine the effect on fertility. Results showed that semen volume, number of spermatozoa per ejaculate and testes weight were not altered by levels of 115, 100, 85, and 70 percent restriction. However adverse effects were noted in birds fed the 55 percent restricted diet. It was suggested that individually caged males require 19.72 g of CP and 1.44 MJ ME/bird/day for BW maintenance.

However, Buckner *et al*. (1986) found that males on a more severe feed restriction of 11.92 g of CP and 1.21 MJ ME/bird/day showed significant reductions in semen volume, number of spermatozoa per ejaculate and testes weight. Cerolini *et al*. (1995) also observed a reduction in reproductive performance of males being fed a restricted diet of 13.2 g CP and 1.27 MJ ME/bird/day during the reproductive period. However, a daily increase in the quantity of feed was seen to improve the fertility of the males. Cerolini *et al*. (1995) suggested a daily feed supply of 15.6 g CP and 1.50 MJ ME/bird/day for best reproductive performance.

Sexton *et al*. (1989a) suggested that reproductive functions of broiler breeder males in cages are more sensitive to under feeding than over feeding. Hocking *et al*. (2002) observed that broiler breeder females on a conventional feed restriction program compared with *ad libitum* feeding showed a decrease in the average daily feed consumption during rearing and early lay and an increase in feed consumption after the peak rate of egg production. The restricted birds had fewer defective or damaged eggshells, higher total egg

production, higher fertility and hatchability than those fed *ad libitum*. Bird mortality was also seen to decrease by more than half.

2.3.3.2 Qualitative Feed Restriction

Despite the positive influences on health and reproduction in broiler breeders, there is some evidence that feed restriction has a negative effect on welfare. As a consequence of being chronically hungry, birds are subject to physiological stress as well as abnormal behaviour (Mench, 2002). Feed restriction causes problems of frustration, particularly seen in individually caged males, resulting in aggression, hyperactivity, pacing before feeding time and pecking at non-feed objects after feeding, which may lead to secondary stress problems, affecting the fertility of the birds (Shea *et al*., 1990; Hocking *et al*., 2001; de Jong *et al*., 2002). Therefore the use of low density diets as an alternative feeding method may improve broiler breeder welfare and therefore subsequent fertility.

To my knowledge there is limited literature on the effects of qualitative feed restriction on fertility in broiler breeder males. Zuidhof *et al*. (1995) found an improvement in the well-being of feed restricted broiler breeder females when fed diets diluted with ground oat hulls from 0 to 56 WOA. Sandilands *et al*. (2005) found that during rearing, broiler breeder females can be successfully limited in growth rates by qualitative feed restriction, and observed that this resulted in significant changes in behaviour that suggests improvements in bird welfare.

2.3.3.3 Separate Sex Feeding

Separate-sex feeding systems are currently being used in the broiler breeder production. A grill covering the female feeder is too fine for the males to access the feed, and the male feeder is placed high enough to prevent the hens from reaching the male feed (Etches, 1996). This permits the males and females to be fed different amounts of feed or different feed rations. Fontana *et al*. (1990) showed that eggs produced in pens where males and females were fed separately allowing the males to be fed a separate diet had a significantly

higher fertility than eggs produced in pens where the males and females received the same breeder diet.

2.4 THE EFFECT OF CRUDE PROTEIN ON MALE FERTILITY MEASURES

There are a number of methods to assess male broiler breeder fertility. Most methods assess semen quality as it is of prime importance with respect to spermatozoa survival *in vivo* or *in vitro* as well as fertilizing ability (de Reviers & Williams, 1984). In previous work, sperm concentration, semen volume, number of spermatozoa per ejaculate, testes weight and spermatozoa viability were used as prime indicators of male broiler breeder fertility (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Wilson *et al*., 1988; Hocking, 1989; Revington *et al*., 1991; Zhang *et al*., 1999). However there are more recent, reliable indicators of male fertility such as sperm mobility (Froman & McLean, 1996) and the sperm-hole assay (Bramwell *et al*., 1995). Some of these measures will be discussed, as well as literature to show where they have been used to assess the effects of dietary crude protein in male broiler breeders.

2.4.1 Semen Collection

The most efficient method for the collection of semen from gallinaceous birds such as chickens is through the abdominal massage technique (Burrows & Quinn, 1937). The reasons why this normal manual manipulation stimulates ejaculation in the rooster is unknown since the technique in no way resembles natural mating (Etches, 1996; Leeson & Summers, 2000). The technique involves holding the male breast down with the feet held at right angles to the body, a second personal then firmly massages the abdomen with one hand while the other hand is firmly drawn across the back in two or more movements (Etches, 1996; Leeson & Summers, 2000). The phallus will then enlarge at which time the collector transfers the hand massaging the back to the cloacal area, placing the thumb and

index finger on either side of the cloaca and gently applying pressure to expel the semen, taking care not to force transparent fluid through the phallic folds (Etches, 1996; Leeson & Summers, 2000). The males will usually not give semen on the first attempt, and they require at least three or four training sessions on alternative days before they will produce clean semen that is free of urates and faeces (Etches, 1996).

2.4.2 Spermatozoon Morphology

Domestic birds have a long, cylindrical sperm cell, with the posterior end merging into a long tapering tail (Jull, 1940; Etches, 1996). As with other animal spermatozoa, avian sperm are comprised of an acrosome, a head, a midpiece and a tail (Etches, 1996). The acrosome is located at the anterior end of the sperm cell and contains proteolytic enzymes which it uses to access the ovum during fertilization. The midpiece and the tail provide motility to the functional spermatozoa. The whole midpiece is surrounded by approximately 30 mitochondria, which provide enzymatic capabilities for energy metabolism.

Morphological abnormalities/defects in the spermatozoa can decrease the fertilizing ability of the sperm (Alkan *et al*., 2002). A number of morphological abnormalities can occur in the acrosome, head, mid-piece or tail regions of poultry spermatozoa (Alkan *et al*., 2002). Some of these abnormalities include bending, coiling, knotting or rounding and swelling of the head, mid-piece and acrosome as well as tail defects (Tsukunaga, 1987; Bekker, 2008). These morphological defects are most frequently observed at the acrosome and mid-piece, suggesting that these areas are most susceptible to environmental factors (Alkan *et al*., 2002). Bekker, (2008) fed broiler breeder males three feed treatments differing only in CP percentage (10.46, 12.61 and 15% CP), and no significant effect of CP level on sperm morphology was seen, however the percentage of normal sperm was seen to decrease with age in all of the CP treatment groups.

2.4.3 Sperm Concentration

There are two ways in which the sperm concentration of semen sample can be determined, either by directly counting of a diluted semen sample with a haemocytometer or by indirect methods, which are more reliable and generally less time consuming (Leeson & Summers, 2000). Brillard & McDaniel (1985) tested the following four methods: haemocytometer, Coulter counter, optical density (spectrophotometer), and spermatocrit for their efficiency to estimate the concentration of spermatozoa in chicken semen. They showed the Coulter counter and optical density methods to be the least time consuming, and concluded that these two methods were also more reliable than either the hemocytometer or spermatocrit methods in estimating semen concentrations in chickens.

No difference in sperm concentration was reported for males fed either 12%, 14%, 16% or 18% CP from 5-53 WOA (Wilson *et al*., 1987a). Wilson *et al*. (1987b) and Wilson *et al*. (1988) also observed no difference in sperm concentration when males were fed either 9%, 12% or 15% CP diets from 7-50 WOA. No difference in sperm concentration was observed when males were fed different CP diets (8 to 40% CP) from 18-64 WOA (Hocking, 1989). Males fed either 8% or 12% CP diets from 24-64 WOA showed no significant difference in sperm concentration, however, males fed the 8% CP diet showed lower than recommended body weights throughout the study (Revington *et al*., 1991). No difference in sperm concentration was reported for males fed either a 12% or 16% CP diet from 21 to 54 WOA (Zhang *et al*., 1999). Hocking & Bernard (1997b) observed that males fed a 16% CP diet had a lower sperm concentration opposed to males fed a 12% CP diet. Sperm concentration in all of the above studies was determined by the optical density method, with the use of a spectrophotometer.

2.4.4 Semen Volume and Number of Spermatozoa per Ejaculate

There are two methods for determining semen volume. The first method is by directly measuring the volume using a measuring cylinder. The second indirect method is by weighing the semen sample and converting it to volume using the specific gravity of semen (1g ≈ 1ml) (Brillard & de Reviers, 1981). The second method is quicker and more precise when measuring smaller volumes of ejaculate. The number of spermatozoa per ejaculate is determined by multiplying the sperm concentration by the semen volume.

Crude protein intake had no effect on semen volume (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Wilson *et al*., 1988; Hocking, 1989; Revington *et al*., 1991). However, Zhang *et al*. (1999) reported higher semen volumes in males fed 12% CP compared to males fed 16% CP from 28-36 WOA. No significant effect of dietary CP was found on the number of sperm ejaculated (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Wilson *et al*., 1988).

2.4.5 Testicular Weight and Function

Testes develop to maximum size once the male birds reach maturity and unless the birds are stressed are unlikely to regress during the breeding period. Therefore, testicular size potentially relates to physiological reproductive status in broiler breeders (McGary *et al*., 2002). A direct relationship has been shown between sperm production and testes size (Ansah *et al*., 1985; Lee *et al*., 1999; Leeson & Summers, 2000). Testicular weight has also shown to be positively correlated with body weight (Brown & McCartney, 1983; Wilson et al., 1988; Fontana *et al*., 1990; Leeson & Summers, 2000). The use of testicular weight as a fertility indicator can be used as a selection tool to improve future flocks, however, evaluation of the testes at the onset of maturation rather than by necropsy at the end of the breeding cycle would be invasive and expensive (McGary *et al*., 2002). Evaluation of the testes is normally performed at the end of the production period by euthanizing the males and removing their testes. No difference in testicular weight at the end of production was observed between males fed 9%, 12% or 15% CP diets (Wilson *et*

al., 1988). Hocking (1989) showed a linear response of plasma uric acid to dietary CP levels above 137 g CP/kg, and suggested that the raised concentration of plasma uric acid which is necessary to excrete the excess nitrogen of protein catabolism has an effect on testicular function. However, some birds are genetically more able to withstand the ill effects of high plasma uric acid concentrations.

2.4.6 Spermatozoa Viability

This is the measure of percentage live spermatozoa in a semen sample, or conversely the measure of percentage dead and abnormal spermatozoa. According to Leeson & Summers (2000) the percentage of dead and abnormal spermatozoa in a sample should be less than 10%; however Wilson *et al*. (1969) reported a significant decrease in fertility when the percentage of dead spermatozoa in a sample was greater than one percent. A common method used to determine sperm viability is with a nigrosin-eosin stain combined with simple microscopic examination (Leeson & Summers, 2000). When stained the live viable spermatozoa do not take up the pink-coloured eosin stain, and therefore remain white on the blue (nigrosin) background. At the same time the dead sperm take up the eosin, and appear pink. A field of view can then be measured for live vs dead sperm when viewed under the microscope at 80-100x magnification (Leeson & Summers, 2000). A trypan blue stain was used by Wilson *et al*. (1969) to measure spermatozoa viability. Live cells remained unstained, while the heads of the dead cells became wholly or partly stained blue. Live abnormal sperm can also be counted when viewing the sample under the microscope. Most abnormal sperm are characterized by severe bending in either the head, mid or tail region, and are distinguishable from the gently curved normal sperm with a symmetrical shaped body culminating in a short thin tail (Leeson & Summers, 2000).

2.4.7 Sperm Penetration Holes in the Inner Perivitelline Layer (IPVL) of the Egg (sperm-hole assay)

Within approximately 15 minutes of ovulation, sperm encounter the ovum in the infundibulum (Wishart & Stains, 1999). Spermatozoa were perceived to merely pass through the fibres of the porous perivitelline layer of the ovum; however Bakst & Howarth (1977) clearly demonstrated through the use of scanning electron microscopy that spermatozoa penetrate the perivitelline layer by enzymatically hydrolysing the underlying fibres. Upon making contact with the inner perivitelline membrane, spermatozoa initiate the acrosomal reaction resulting in the release of acrosomal proteases which hydrolyse a small hole in the membrane allowing the spermatozoon to enter and fertilize the ovum (Howarth, 1983).

Wishart (1987) was the first to demonstrate the quantitative relationship between fertility in chickens and the number of spermatozoa which interact with the egg, by showing a correlation between the numbers of spermatozoa trapped in the outer perivitelline layer (OPVL) with egg fertility and the length of the fertile period of artificially inseminated hens. However measuring sperm trapped in the OPVL is not the best method of estimating fertility or breeding efficiency in birds, because hundreds of sperm may be found in the OPVL of eggs, but may not necessarily have resulted in fertilization (Stains *et al*., 1998). An alternative method for estimating the sperm-egg interaction was suggested by Bramwell & Howarth (1992) involving the quantification of holes hydrolysed by spermatozoa as they penetrate the IPVL of the egg. In eggs, there is a concentration of holes in the IPVL over the germinal disc area (Bramwell & Howarth, 1992). These holes can be viewed in samples of perivitelline membrane from oviposited eggs stained with Schiff's reagent under microscopy at low power (Birkhead *et al*., 1993). Counts of the holes around the germinal disk in such samples are representative of the numbers of sperm interacting with the egg at fertilization, and therefore can be related to fertility of the eggs (Bramwell *et al*., 1995; Stains *et al*., 1998). A mean number of three holes in the IPVL

over the germinal disk region coincided with a mean fertility of 75% (Bramwell *et al*., 1995). Wishart (1997) showed that eggs had a 50% probability of being fertile if three or more spermatozoa penetrated the IPVL around the germinal disk region, and showed maximum fertility when more than six spermatozoa penetrated the IPVL around this region. Staines *et al*. (1998) suggested that counting holes in the IPVL of laid eggs is a more convenient method for assessing breeding efficiency and predicting flock fertility than counting spermatozoa trapped in the OPVL.

2.4.8 Sperm Mobility

The use of spectrophotometry has been reviewed to be one of the four principle methods which can be used to objectively measure the mobility of poultry semen (Froman & McLean, 1996). Previous methods were based on sperm cells being able to move in a direction against the current of a fluid (Wall & Boone, 1973). However Froman & McLean (1996) used the net movement of fowl sperm into 6% (w/v) Accudenz® to develop a sperm mobility assay. Accudenz® is a nonionic, biologically inert cell separation medium which is typically used as a medium for density gradient centrifugation (Froman & Feltmann, 1998). When a sperm suspension is overlaid on the Accudenz medium, a distinct interface forms because of the difference in density between the two media (Froman & Feltmann, 2000). After a short incubation interval and through the use of spectrophotometry Froman & McLean (1996) were able to quantify the movement of sperm into the Accudenz® and thus provide a means of measuring sperm mobility. Unlike sperm motility, in which the percentage of moving sperm cell are estimated, sperm mobility is a measure of the proportion of sperm in a semen sample with a powerful and relatively linear forward motion (Donoghue, 1999).

Froman & Feltmann (1998) confirmed, using a base population of 271 roosters, that fowl sperm mobility, as measured using the sperm mobility assay, is a normally distributed variable. This sperm penetration assay has been used to diagnose heritable subfertility in roosters (McLean & Froman, 1996). Froman & Feltmann (1998) showed that *in vitro*

measurements of fowl sperm mobility were relevant to reproductive efficiency. It has also been shown over a long-term fertility trial that selection of semen donors based upon sperm penetration of Accudenz® increased the number of progeny obtained from hens (Froman *et al*., 1997). This was corroborated in a later study by Froman & Feltmann (1998) who observed a 10% improvement in hatchability by selecting semen donors based upon higher sperm mobility. A high correlation between sperm ATP content and sperm mobility was also observed, indicating that sperm able to produce more ATP showed a higher mobility (Froman & Feltmann, 1998).

Froman & Feltmann (1998) observed extreme differences in sperm mobility within a population. For example, 95% of sperm will enter the Accudenz® solution in the case of some males, whereas less than 5% will enter in the case of others. Prior to ejaculation, sperm cells in the rooster's deferent duct are immotile. Therefore at this time there is no variation in sperm cell motion among males (Froman & Feltmann, 1998). However after ejaculation there are profound differences in sperm cell motion among males. The motion of a sperm cell is dependent on its physical environment as well as its chemical environment, temperature, structural integrity and metabolic capacity (Froman & Feltmann, 1998). "Therefore, phenotypic differences in sperm mobility may be explicable in terms of how sperm react to the interface between the sperm suspension overlay and the Accudenz solution" (Froman & Feltmann, 1998). Froman *et al*. (1999) measured the linear velocity and trajectory (straightness) of freshly ejaculated sperm from average mobility males and high-mobility males. Both variables were significantly higher ($P<0.001$) in the high mobility phenotype males. The two variables were also found to be highly correlated ($r = 0.9$). This was corroborated in a later study by Froman & Feltmann (2000). This provided an explanation for phenotypic differences in sperm mobility, and led to the theoretical basis for the relationship between sperm mobility and male fertility.

Froman *et al*. (1999) measured the sperm mobility of 48 broiler breeder males using the sperm mobility assay and plotted their absorbance values against their fertility. Fertility in this case was measured by inseminating 8-12 hens per male, collecting the eggs over seven days following AI, and examining the egg contents after four days of incubation. From this

they were able to show that differences in fertilizing ability among males are explained by sperm mobility, and concluded that fertility in broiler breeders is more likely determined by characteristics associated with sperm, than selection exerted by the oviduct of the female. Although there are a number of important factors which play roles in male fertilizing capacity, such as the sperm-egg interaction, it appears that sperm mobility is the most important attribute (Froman *et al*, 1999).

Froman & Feltmann (1998) attributed the predictive value of the sperm penetration assay to the following facts. Firstly sperm cells must be motile in order to ascend the vagina and enter the sperm storage tubules. Secondly if you place immotile sperm in the hen's oviduct they are able to fertilize the oocytes. Thirdly unlike mammalian sperm, fowl sperm does not go through capacitation. "Therefore, the sperm penetration assay simulates a critical step for internal fertilization in the hen: the net movement of sperm cells, presumably against a resistance" (Froman & Feltmann, 1998).

Sperm mobility does not appear to be correlated with BW or testes weight (Bowling *et al*., 2003). A study by Holsberger *et al*. (1998) showed no relationship between sperm mobility phenotype and sperm concentration, semen volume, sperm viability or sperm membrane activity. The sperm mobility phenotypes however, remained consistent with the high mobility toms remaining high and the low mobility toms remaining low over the five month trial. Donoghue (1999) concluded that the sperm mobility test, the sperm-hole assay, and the sperm binding assay with sperm from individual roosters and toms are predictive of fertility, and can be used as a management tool for sire selection.. Froman *et al*. (2002) showed sperm mobility to be a heritable trait, however there is a possibility that it is under the control of an independent, maternally inherited element, suggesting that "mothers may have an important genetic influence over the sperm mobility of their sons". It was suggested that mothers may influence sperm mobility through the X chromosome or mitochondrial DNA, however if the latter is true males are passive carriers of mitochondrial DNA which is exclusively maternally transmitted, meaning the extent to which males control sperm mobility is effectively at a 'dead end' (Froman *et al*., 2002).

To date and to the best of my knowledge there has been no use of mobility to assess the influence of environmental factors such as CP intake. However due to the strong genetic contribution, mobility may be unlikely to be affected by environmental factors (P = G + E).

2.5 CONCLUSIONS

Broiler breeders have the same rapid growth genes as their offspring, however if allowed to eat *ad libitum* like their offspring, a number of complication may occur. Body weight would increase rapidly too early on in their life resulting in impaired health, mating ability and decreased fertility. Feed restriction programs are put into place to maintain BWs and flock fertility. Crude protein level in the feed plays an important role in maintaining flock fertility during the production phase, however male and female broiler breeders require different amounts of CP due to the different reproductive processes which utilize amino acids. Males are often fed a female ration which is higher in CP than what the male requires, and due to the high cost of protein in feed as well as the potential negative effects on fertility reported in the literature, assessing the optimum CP intake in the current strains of male broiler breeders is essential. However, feeding lower amounts of CP to males may have a negative effect on their fertility, and it may also be difficult to formulate rations low in CP. A number of techniques used to measure male fertility have been discussed, including some more recent techniques, such as sperm penetration of the IPVL of oviposited eggs and sperm mobility, which have been shown to be highly correlated to male fertility, in comparison to other measures of fertility more traditionally used.

Therefore the aim of this study was to employ these more recent techniques to assess male fertility, as well as some of the more traditional measures to determine the effects of CP intake on male broiler breeder fertility at different ages.

CHAPTER 3

THE EFFECT OF DIETARY CRUDE PROTEIN INTAKE ON FERTILITY IN YOUNG AND OLD MALE BROILER BREEDERS

3.1 INTRODUCTION

Body weight management and proper feeding strategies for broiler breeders is of great importance. The growth of broiler breeders needs to be limited in order to maintain or improve fertility (Mench, 2002). The protein requirements for broiler breeders are likely to change due to the continuous genetic improvements in the broiler industry as well as the need for feed restrictions (Barbato, 1999). Previous research has suggested that breeders can be fed lower crude protein (CP) diets as a means of moderating their growth rate with no adverse effects on reproductive performance (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Revington *et al*., 1991; Lopez & Leeson, 1995; Leeson & Summers, 2001) and have identified a 12% dietary CP level as optimal, which equates to a CP intake of 12.63-15.6 g/bird/day (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Hocking, 1989; Revington *et al*., 1991).

However, results from experiments that have been conducted on this subject have not been consistent. In some cases there was no significant effect of dietary CP content on sperm concentration (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Wilson *et al*., 1988; Hocking, 1989; Revington *et al*., 1991; Zang *et al*., 1999), semen volume (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Wilson *et al*., 1988; Hocking, 1989; Revington *et al*., 1991), number of spermatozoa per ejaculate (Wilson *et al*., 1987a; Wilson *et al*., 1987b), the metabolic activity of spermatozoa (Hocking, 1989), testes weight (Wilson *et al*., 1987a; Wilson *et al*., 1987b) or fertility (measured as fertile eggs candled at 18 days of incubation per total eggs

set) (Fontana *et al*., 1990; Hocking & Bernard, 1997a). However, others have reported that a high CP diet adversely affected the concentration of spermatozoa (Hocking & Bernard, 1997b), semen volume (Zhang *et al*., 1999), the proportion of males producing semen (Wilson *et al*., 1987a; Hocking, 1989) and fertility (measured as fertile eggs candled at 18 days of incubation per total eggs set) (Hocking, 1990). Other reports show that a low CP diet increased carcass fat; this is most likely due to the diets being isocaloric and the CP to energy content not being balanced (Wilson *et al*., 1987b; Revington *et al*., 1991). Borges *et al*, (2006a) also observed an increase in carcass crude fat percentage at extreme levels (deficiency or excess) of protein intake, as well as a decrease in reproductive performance, and recommended a CP intake of 16.9 g/bird/day to meet the CP requirements of male broiler breeder from 27 to 61 WOA.

Assessing the optimum levels of CP required for maximum male fertility can be beneficial in several ways. As one of the most costly components of poultry feed, overfeeding or incorrect feeding of protein has several downsides. Protein which is not utilized in body gains or production cannot be stored and has to be converted into a non-toxic metabolite, uric acid, and eliminated from the body (Coon, 2004). The conversion to uric acid requires a significant amount of metabolic energy and could thus result in a relative energy deficiency (Hocking, 1989). Poor quality dietary proteins and amino acids can cause heat stress problems for animals due to the inefficient process of incorporating feed proteins and amino acids into body proteins which has a high heat increment from metabolism (Coon, 2004). Therefore poor quality dietary proteins and amino acids could intensify heat stress conditions in poultry housed in hot climates, resulting in a significant reduction in performance (Coon, 2004). There are also environmental concerns about nitrogen losses in animal waste and the resultant negative impact on fresh water supplies. Broiler breeder diets vary in their protein and amino acid contents, which have been observed to affect the nitrogen content of the excreta; this was found to increase with increasing CP contents of the diets (Lopez & Leeson, 1995).

The nutrition of male breeders is often overlooked, and males are often fed a female diet which is high in protein. A high protein (female breeder) diet fed to the males during the

breeding period was associated with a decline in fertility from 45 to 60 WOA compared with that of males fed a low protein diet (Hocking, 1990). Due to mating behaviour and requirements, the impact of sub-fertile males in the overall fertility of the flock could be large (Hazary *et al*., 2001; McGary *et al*., 2003). Maximizing male fertility could ensure maximizing fertile egg production, which would result in more broiler chicks without increasing the size of the breeding flock. Small improvements in management could greatly improve male performance.

The importance of feeding the correct amount of CP to breeder males has been discussed, and it is evident that there are some discrepancies with regard to the influence of various levels of CP on the reproductive performance of the males. Therefore, the objective of this experiment was to observe the effect of three different dietary CP levels on the sperm concentration, sperm mobility and sperm penetration holes of the IPVL of oviposited eggs in young and old male broiler breeders in the production phase, and to determine the optimum level of dietary CP to achieve maximum fertility.

3.2 MATERIALS AND METHODS

One hundred and forty four Ross 308 broiler breeder males, 72 at 27 WOA (young) and 72 at 52 WOA (old) were placed in individual cages (60cm wide x 44cm deep x 60cm high) in one of 6 light-tight rooms (12 birds per room) at Ukulinga research farm, Pietermaritzburg. Birds were provided with a 13 h photoperiod (06:00 to 19:00).

Prior to the start of the experiment (20 – 27 WOA for the young birds and 35 – 52 WOA for the old birds) a commercial breeder feed (130 g CP/kg, 11.7 MJ/kg) was provided. The birds were weighed individually every two weeks and the feed allocation for the young and old groups of males adjusted to adhere to the growth curve recommended by the primary breeder. During this time birds were trained to produce a semen sample (Burrows and Quinn, 1937), which was analyzed for mobility (Froman & McLean, 1996) using a Turkey Mobility Analyser 591B (ARS, Chino, CA), calibrated for use in chickens. The 72 young

males were selected from a group of 79 and the 72 old males from a group of 122. Those with higher (but still variable) mobility scores with a BW close to that recommended by the primary breeder were selected, in order to have an even distribution among mobility phenotypes. Males in each age group were ranked and placed in rooms in order of rank.

The birds were provided with *ad libitum* access to water throughout the experimental period and allocated to one of three dietary treatments (a low, medium or high CP diet). Two isocaloric diets (11.7 MJ/kg) were formulated to contain 100 g CP/kg (low protein) and 140 g CP/kg (high protein). Except for the CP, both diets were formulated according to the breeder nutrient specifications of a male breeder feed from 24 weeks onwards (Arbor-Acres-Plus-Avigen, 2007). The ingredient contents of the two diets can be found in Table 3.1. A third intermediate diet was then blended from the high and low protein diets to contain 120 g CP/kg (medium protein). A sample of each experimental diet was then analyzed for their respective nutrient compositions (Table 3.1).

Table 3.1 *The ingredient and nutrient contents (units specified) of the low, medium and high protein diets*

Ingredient	Unit	Low	Medium	High
Maize	g/kg	704		645
Wheat bran	g/kg	169		200
Soya bean 46	g/kg			118
Sunflower 34	g/kg	39.1		
L-lysine HCL	g/kg	0.90		0.06
DL methionine	g/kg			0.07
Vitamin & mineral premix	g/kg	2.50		2.50
Filler (sand)	g/kg	40.0		
Limestone	g/kg	19.7		19.5
Salt	g/kg	1.47		2.67
Sodium bicarbonate	g/kg	5.60		1.27
Oil- sunflower	g/kg	8.85		7.88

Kynofos	g/kg	8.95		3.86
Nutrient composition (as is)				
AMEn adult	MJ/kg	12.3	12.2	12.2
Crude protein	%	9.68	11.7	13.0
Dry matter	%	89.1	88.9	88.4
Moisture	%	10.9	11.1	11.6
Calcium	%	1.95	0.85	0.88
Phosphorous (total)	%	0.69	0.52	0.58
Methionine	%	0.18	0.21	0.23
Cysteine	%	0.21	0.23	0.26
Methionine + Cysteine	%	0.39	0.44	0.49
Lysine	%	0.37	0.51	0.61
Threonine	%	0.34	0.42	0.49
Arginine	%	0.57	0.72	0.84
Isoleucine	%	0.33	0.43	0.50
Leucine	%	0.94	1.09	1.17
Valine	%	0.45	0.55	0.63
Histidine	%	0.25	0.31	0.35
Phenylalanine	%	0.46	0.56	0.63
Glycine	%	0.44	0.52	0.58
Serine	%	0.45	0.55	0.63
Proline	%	0.73	0.83	0.93
Alanine	%	0.60	0.68	0.73
Aspartic acid	%	0.69	0.95	1.15
Glutamic acid	%	1.80	2.14	2.40

The methods used to determine nutrient compositions of the experimental feeds were from McNab & Fisher (1984) for AMEn (MJ/kg), the Association of Official Analytical Chemists (AOAC) Official Method 990.03 (1995) for crude protein and Moore & Stein (1948) for amino acids.

Each feed treatment was randomly allocated to 4 of the 12 birds in each room. The young males received a feed intake of 130 g/bird/day from 27-41 WOA (phase 1) and thereafter from 42-60 WOA (phase 2) the feed allocation was increased to 142 g/bird/day. The old males received a feed intake of 145 g/bird/day for 11 weeks until 63 WOA. The daily (Table 3.2) and cumulative (Table 3.3) CP intakes were calculated for males at different ages on each treatment.

Table 3.2 *The CP intakes of young and old males fed the low medium and high protein diets*

	Crude protein intake (g/bird/day)		
	Low	Medium	High
Young males (phase 1)	12.6	15.2	16.9
Young males (phase 2)	13.7	16.6	18.5
Old males	14.0	17.0	18.9

Table 3.3 *The cumulative CP intakes of young and old males fed the low, medium and high protein diets as well prior to the experiment*

	Cumulative protein intake (g CP)		
	Low	Medium	High
Young males (phase 1)	1234.8	1489.6	1656.2
Young males (phase 2)	1822.1	2207.8	2460.5
Total (phase 1 & 2)	3056.9	3697.4	4116.7
Old males	784	952	1058.4

3.2.1 Fertility Measurements

Males were trained for semen collection three times a week prior to, and once a week during the experimental period. Semen samples were collected for analysis at 27, 29, 32,

35, 41, 47, 52, 55, 58 and 60 WOA for the young males and at 52, 54, 57 and 60 WOA for the old males. The feathers around the cloaca of each male were plucked in order to minimize contamination of the semen during collection. To prevent dilution of the sample, care was taken to avoid collecting the transparent watery fluid that is sometimes produced by the fowl at ejaculation in response to massage (Lake & Stewart, 1978). The semen from males on the same treatment in each room was pooled prior to analysis (pooled sample from each treatment is the experimental unit).

Each pooled sample of semen was tested for sperm concentration, sperm mobility and was used to artificially inseminate commercial Lohmann egg-type hens (37 WOA at first insemination) housed in cages (50cm wide x 50cm deep x 50cm high) containing either two or three birds per cage. Eggs were collected on days 2, 5 and 8 post-artificial insemination (PAI) and stored in a cold room for no more than two weeks before being assessed for the number of points of sperm hydrolysis in the inner perivitelline layer (IPVL).

3.2.1.1. Sperm concentration

Immediately after semen collection, 11.4µl of neat semen from each sample was diluted with 3.42ml of 3% (w:v) NaCL in a disposable cuvette. Concentration was determined by measuring absorbance at 550nm using a Turkey Mobility Analyser Model 591B (Animal Reproduction Systems, Chino, CA), calibrated for use in chickens (Howarth, 1995).

3.2.1.2 Sperm mobility

After the concentration of the sample was determined, 50µl of neat semen was diluted to contain 5×10^8 sperm per millilitre with 50mM *N*-tris[hydroxymethyl] methyl-2-amino-ethanesulfonic acid (TES), pH 7.4, containing 120mM NaCl, 10mM glucose, and 2mM $CaCl_2$ (Animal Reproductive Systems, Chino, CA). A 300µl volume of this sperm suspension was then carefully overlaid upon 3ml of pre-warmed 3% (w:v) Accudenz (Accurate Chemical & Scientific Corporation, Westbury, NY) in a disposable cuvette and

incubated at 41°C for 5 min, after which the absorbance of the solution below the layer was measured at 550nm (Froman & McLean, 1996)

3.2.1.3 IPVL sperm holes

A 300µl volume of pure semen was diluted to contain 100 million sperm per ml with 50mM *N*-tris[hydroxymethyl] methyl-2-amino-ethanesulfonic acid (TES), pH 7.4, containing 120mM NaCl, 10mM glucose, and 2mM $CaCl_2$ (Animal Reproductive Systems, Chino, CA), to be used for insemination. Artificial insemination was performed in the late afternoon (Donoghue *et al*., 1995; Brillard, 2003). Each hen was held in a similar way to that of the male during semen collection. Gentle pressure was applied in a posterior direction to the abdomen of the hen using the left hand while the tail was pressed in an anterior direction by the right hand, causing the cloaca of the hen to evert exposing the entry to the vagina (Etches, 1996; Leeson & Summers, 2000). One hundred and eighty layer hens were inseminated after each collection (5 hens per treatment). Each hen was inseminated using an Eppendorf pipette with a 50µl volume of the diluted semen sample, containing five million sperm. The diluted semen was inseminated directly into the vaginal orifice of the hen at which point the pressure on the abdomen was relaxed allowing the cloaca to return to its natural position and the pipette gently withdrawn. A new pipette tip was used after each insemination in order to prevent any contamination of semen samples as well as ensuring the general hygiene of the hens.

After the eggs were collected on day 2, 5 and 8 PAI, each egg was cracked open and the yolk and albumen separated. The yolk was then placed on a paper towel and the excess albumen removed by rolling the yolk around. A square of approximately 1 × 1cm of the IPVL situated around the germinal disk area was then cut and removed. The piece of membrane was rinsed in phosphate-buffered saline (PBS) to remove adherent yolk, before being stretched out on a glass microscope slide. A few drops of 5% formalin were placed onto the membrane, after which it was stained with Schiff's reagent and covered with a cover slip. The slides were examined on the same day they were prepared using a light microscope (4 × magnification) and captured with a digital camera. Each image was then

analysed using ImageJ, an image analysis software program (Rasband, 1997) and the number of sperm penetration holes in the IPVL counted in a 4mm^2 area surrounding the germinal disk (Bramwell *et al*., 1995). (Figure 3.1)

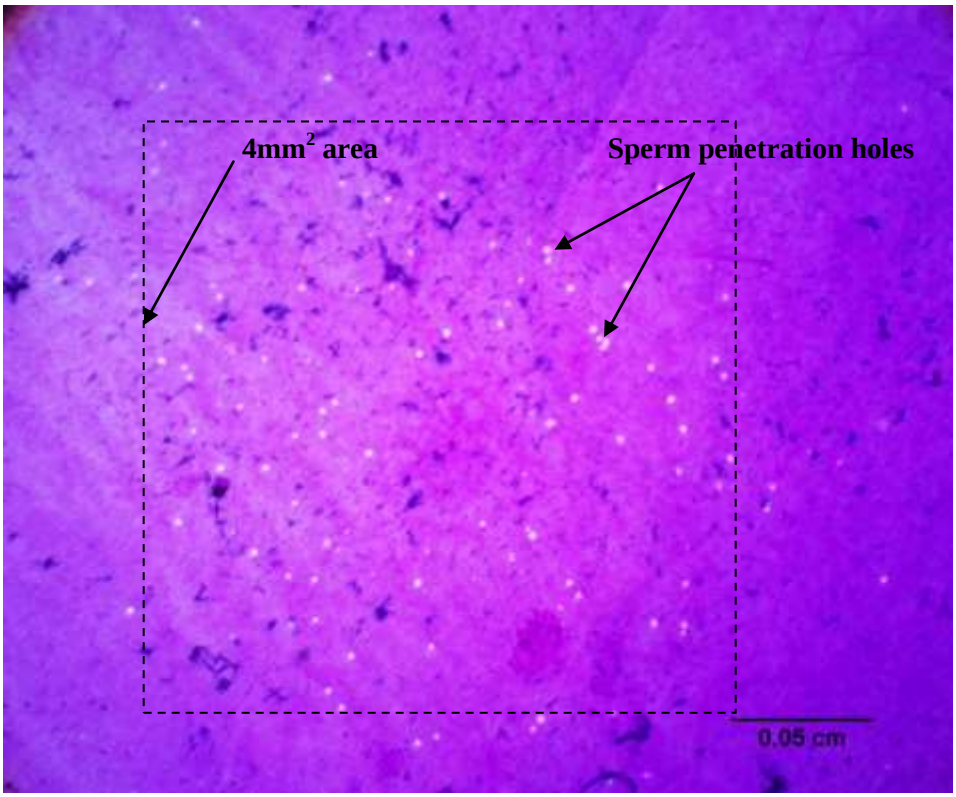

Figure 3.1 *Sperm penetration holes in the IPVL over the germinal disc (4 x magnification) of an oviposited egg stained with Schiff's reagent, with a 4mm^2 area around the germinal disk.*

3.2.1.4 Egg fertility

Wishart, (1997) showed that eggs had a 50% probability of being fertile if three or more spermatozoa penetrated the IPVL around the germinal disk region, and showed maximum fertility when more than six spermatozoa penetrated the IPVL around this region, so the fertility of each egg, based on the number of sperm holes, was predicted at 50% (≥3 sperm holes) and maximum probability (> 6 sperm holes).

3.2.1.5 Testes weight

At 63 WOA the old males were euthanized and the testes removed and weighed.

3.2.2. Statistical Analysis

Unless stated otherwise, all data were analyzed using *GenStat 14th Edition* (VSN International Ltd., 2013). Simple linear regression was performed to determine the response in BW, sperm concentration, sperm mobility and IPVL sperm penetration holes in males of different ages to CP intake. The IPVL sperm penetration hole data needed to be log transformed as it was not normally distributed (confirmed by the Shapiro-Wilks test for normality). Simple linear regression was also performed to determine the response in BW, sperm concentration, sperm mobility and sperm penetration holes to age as well as the response in IPVL sperm penetration holes with days PAI.

A general ANOVA was performed in order to compare BWs between CP treatments for the young and old males.

Simple linear regression was performed on the sperm concentration and mobility data at each collection time and no significant response was seen at any of the collection times,

therefore the values of different collection times were combined for the young males (phase 1), young males (phase 2) and old males.

Sperm concentration and sperm mobility values from the young males (phase 2) and old males were compared with a general ANOVA.

Sperm concentration, sperm mobility and the log number of IPVL sperm holes from the low, medium and high cumulative protein intakes between the young and old males at 60 WOA were compared with a general ANOVA.

A Pearson Chi-Square analysis (SPSS) of fertile and non-fertile eggs was performed in order to asses if CP intake had an effect on egg fertility.

The average testes weights from birds on each treatment were compared with a general ANOVA. The left and right testes weights on each treatment were compared by performing a paired T-test. This was done in order to test the null hypothesis that the mean left testis weight minus the mean right testis weight is equal to zero.

3.3 RESULTS & DISCUSSION

3.3.1. Body Weight

Overall, there was no significant response seen in BW to CP intake in both phases in the young males as well as in the old males. However, at 40 WOA the young males phase 1 showed a significant ($P<0.05$) difference in BWs between CP treatments, with birds on the high CP treatment having significantly lower mean BWs than the birds on the low and medium treatments. On further analysis it was seen that around 32 WOA the average BW of the young males on the high CP treatment began to decrease and diverge from the average BW of the males on the low and medium CP treatments. At 41 WOA when the feed intake of the males was increased from 130 g/bird/day to 142 g/bird/day the average BW of the males on the high CP treatment then began to increase again and over time looks to re-join the average BW's of the males on the low and medium CP treatments

(Figure 3.2). When the feed intake of the males was increased a new feeding cup was made to contain 142 g of the high CP feed, perhaps a problem occurred with the previous feed cup for the high CP feed at 32 WOA resulting in the males not receiving the right amount of feed and once the new feed cup was made containing the correct amount of feed the males were able to once again gain BW. Previous research has shown that birds consuming higher levels of CP showed greater BWs then those consuming lower levels of CP (Wilson *et al*., 1987b; Emmerson, 1997; Zang *et al*., 1999; Romero-Sanchez *et al*., 2008). There was no significant difference in BWs between CP treatments in the old males. This was also observed by Borges *et al*. (2006a). The old males were only subject to the CP treatments for a short period of time compared to the young males, which may not have been long enough for the treatments to have a significant effect on their BW. When BW is controlled by feed allocation, an optimum exists for maximum fertility.

As the age of a flock increases so does the optimum BW, also resulting in an increase in the differential between the fertility of males near the optimum BW and those which are either too small or too heavy (Hocking, 1990). This may help explain the decline in fertility seen in older flocks, there may have been a decrease in flock uniformity with fewer males being near the optimum BW thus resulting in a decrease in flock fertility. The control of male BWs by separate-sex feeding can lead to a significant improvement in fertility at all ages. With the exception of the young males on the high CP treatment between 32 and 40 WOA, all other males gained weight over the experimental period (23 and 10 weeks for the young and old males respectively) and the average bird weight remained close to the growth curve recommended by the primary breeder. The decrease in weight of young males on the high CP treatment is suspected to be due to feeding error. This was corrected and the birds once again began to gain weight and return to the recommended growth curve.

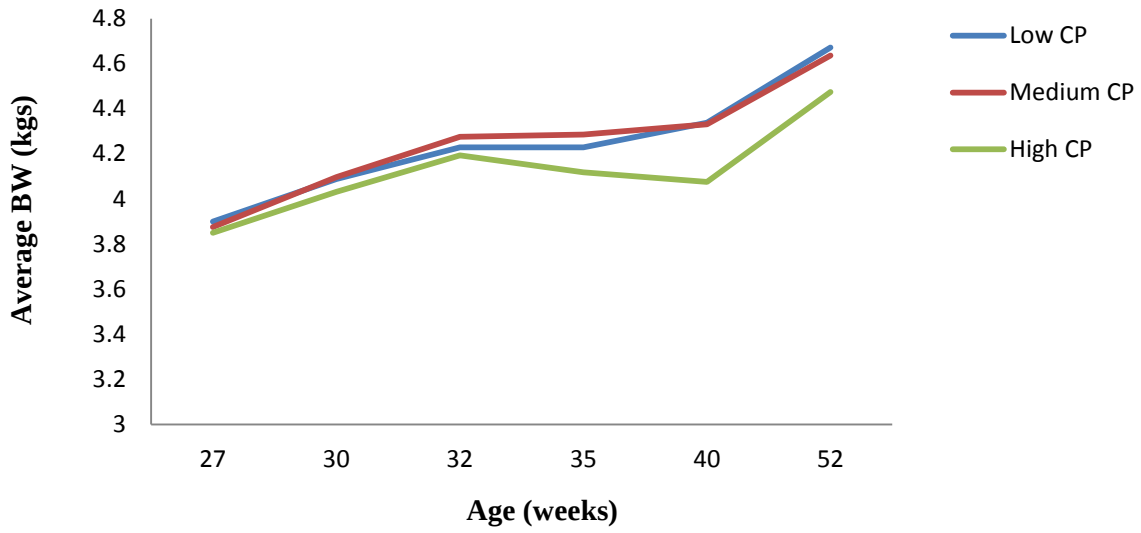

Figure 3.2 *Average BW of young males on the low, medium and high CP treatments with age.*

3.3.2. Sperm Concentration and Mobility

There was no significant response in sperm concentration or sperm mobility to CP intake or age in both the young and old males. Values for sperm concentration and sperm mobility are shown in Table 3.3. A number of authors also observed no significant effect of dietary CP level on sperm concentration (Wilson *et al*., 1987a; Wilson *et al*., 1987b; Wilson *et al*., 1988; Hocking, 1989; Revington *et al*., 1991; Zang *et al*., 1999). Although there was no significant difference between treatments, sperm concentration was seen to be highest in the medium CP treatment for the young males and in the low CP treatment for the old males. This is similar to the observations of Hocking & Bernard (1997b), who observed significantly ($P<0.05$) higher semen concentrations in males on a lower CP diet (120 g CP/kg) than males on a high CP diet (160 g CP/kg). Zang *et al*. (1999) showed over all ages (28-54 WOA) that males consuming a 120 g CP/kg had a numerically but not significantly higher sperm concentration than those consuming 160 g CP/kg. Broiler breeder diets in the range of 150 to 180 g CP/kg clearly have an effect on spermatogenesis,

with a decrease in the proportion of males producing semen, resulting in decreased spermatozoa production and total semen volume (Wilson et al., 1988; Hocking, 1989). Hocking (1989) concluded there to be an absence of any effect of dietary CP on semen/spermatozoal quality, and that if there is an effect of dietary CP on fertility, it is most likely attributable to its effect on the proportion of competent males rather than via an effect on semen production and quality *per se*.

Sperm mobility was seen to be highest for the medium CP treatment in all groups of birds, although this was not a significant response. To date and to the best of my knowledge there has been no literature involving the measure of sperm mobility to assess the influence of environmental factors such as CP intake in broiler breeder males. The expressed phenotype (sperm mobility) has a strong genetic component with a high heritability. Sperm mobility was not affected by the CP level of the dietary treatments; however the CP level may not have been severe enough to influence this trait.

Table 3.3 *Mean values of sperm concentration and sperm mobility (± s.e.m.) measured in the old males (52, 54, 57and 60 WOA), young males phase 1 (27, 29, 32, 35, 41 WOA) and phase 2 (47, 52, 55, 58 and 60 WOA) fed different dietary CP levels*

	Old males		**Young males**			
			Phase 1		Phase 2	
Treatment (CP level)	Concentration sperm/ml ($\times 10^6$)	Mobility %	Concentration sperm/ml ($\times 10^6$)	Mobility %	Concentration sperm/ml ($\times 10^6$)	Mobility %
Low	5.64 ± 0.18	21.29 ± 2.16	5.48 ± 0.10	21.42 ± 1.32	5.36 ± 0.15	23.67 ± 1.94
Medium	5.58 ± 0.15	29.17 ± 2.10	5.58 ± 0.10	23.68 ± 1.51	5.60 ± 0.15	25.13 ± 2.21
High	5.56 ± 0.17	19 ± 2.42	5.46 ± 0.09	19.83 ± 1.25	5.29 ± 0.15	21.83 ± 1.93

3.3.3. IPVL Sperm Holes

The young males (phase 1) showed no significant response in the log number of IPVL sperm holes with increasing CP intake, however a significant ($P<0.001$) positive response was observed in the log number of IPVL sperm holes with increasing CP intake in the young males (phase 2) (Figure 3.3) as well as the old males (Figure 3.4).

The young males (phase 1) showed no significant response in the log number of IPVL sperm holes with CP intake in eggs collected on any of the days PAI. Young males (phase 2) showed a significant ($P<0.05$) positive response in the log number of IPVL sperm holes with increasing CP intake on days two (Figure 3.5) and five (Figure 3.6) PAI, but no significant response in eggs collected eight days PAI. The old males showed a significant positive response in the log number of IPVL sperm holes in eggs collected two days PAI ($P<0.05$) (Figure 3.7) and eight days PAI ($P<0.001$) (Figure 3.8) but no response was seen in eggs collected five days PAI.

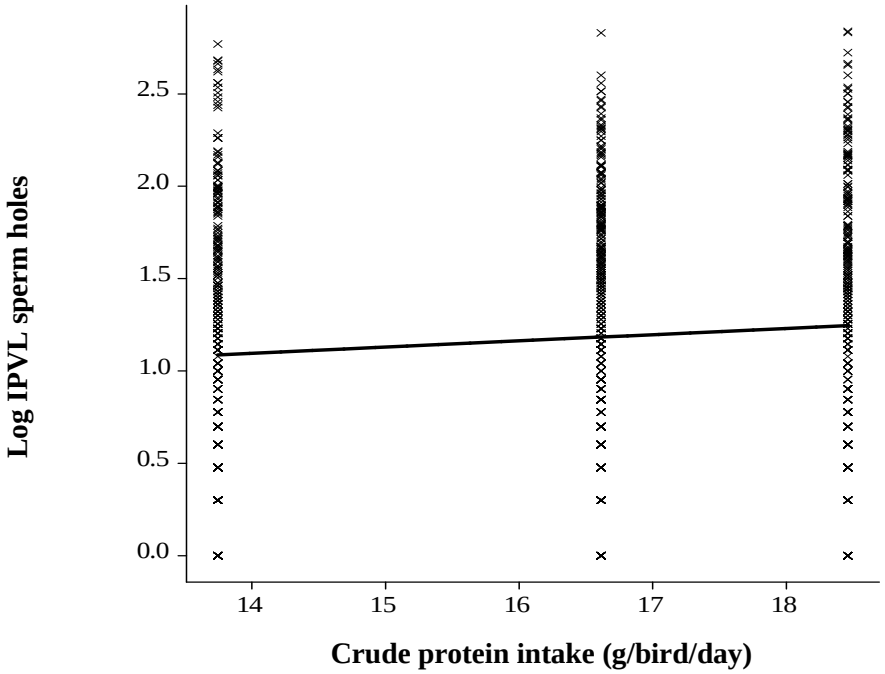

Figure 3.3 *Log number of IPVL sperm holes with increasing CP intake in young males (phase 2).*

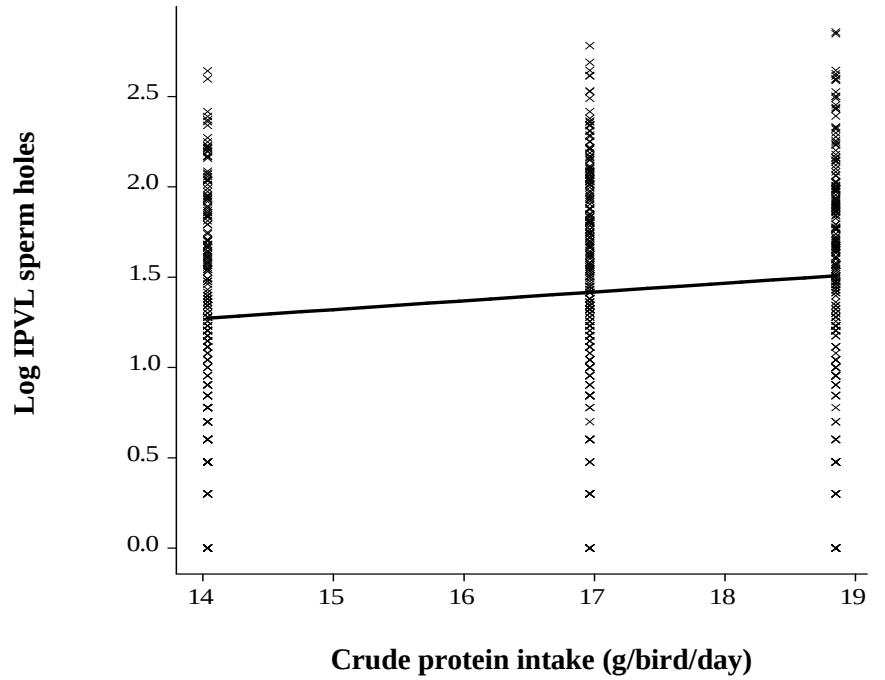

Figure 3.4 *Log number of IPVL sperm holes with increasing CP intake in old males*

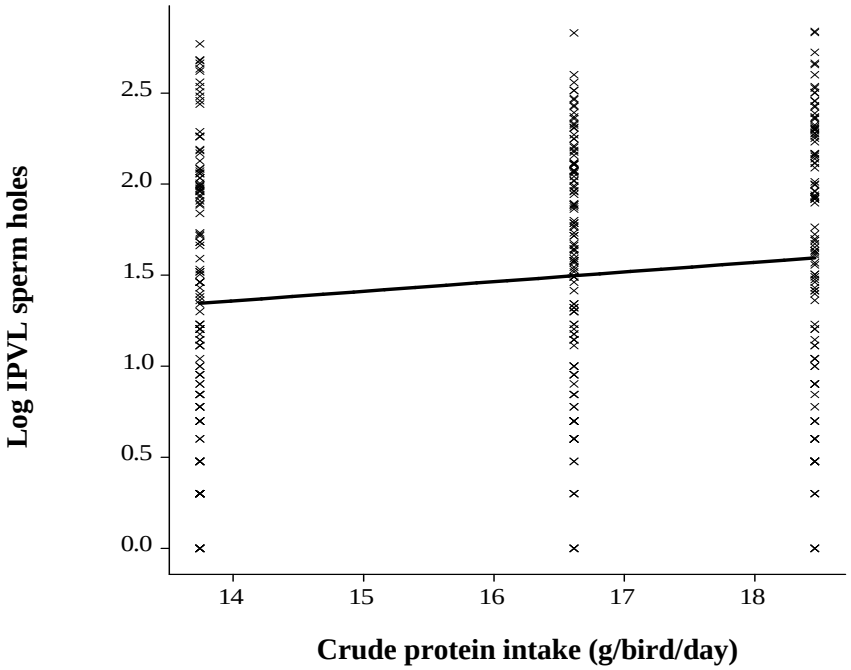

Figure 3.5 *Log number of IPVL sperm holes from eggs collected two days PAI with increasing CP intake in young males (phase 2)*

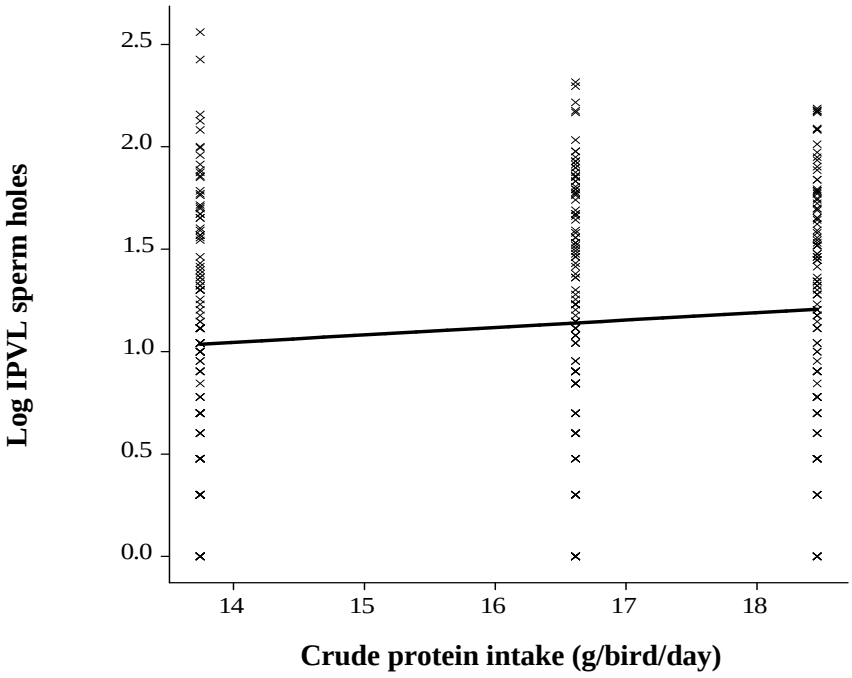

Figure 3.6 *Log number of IPVL sperm holes from eggs collected five days PAI with increasing CP intake in young males (phase 2)*

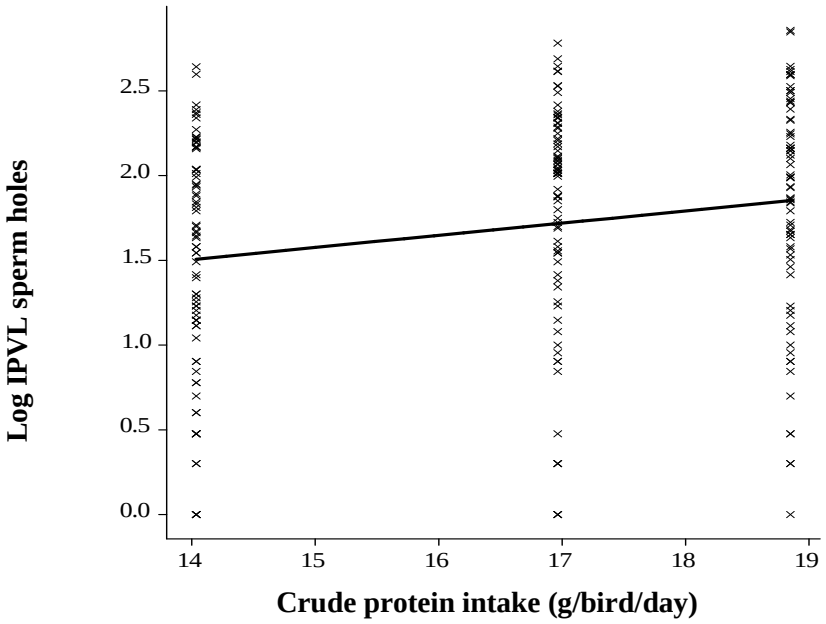

Figure 3.7 *Log number of IPVL sperm holes from eggs collected two days PAI with increasing CP intake in old males*

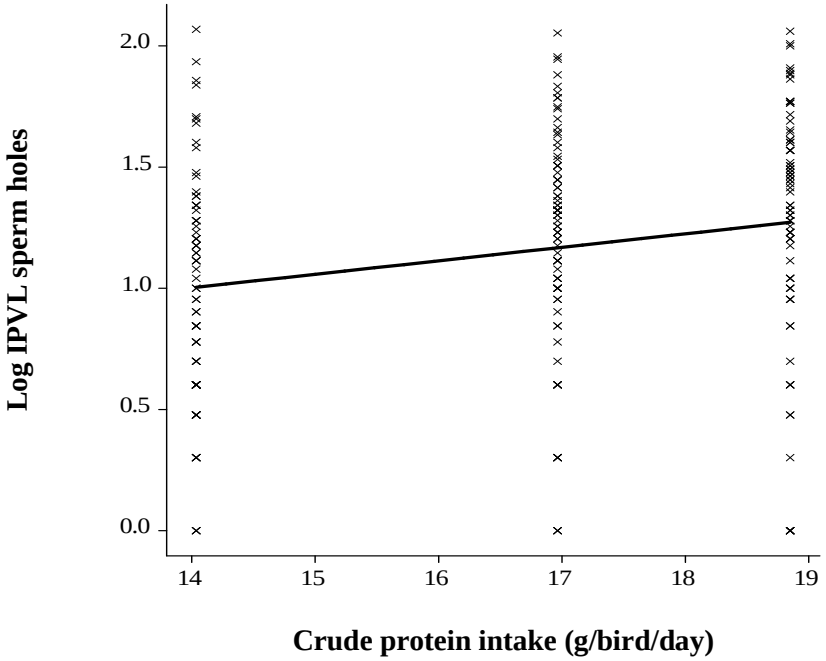

Figure 3.8 *Log number of IPVL sperm holes from eggs collected eight days PAI with increasing CP intake in old males*

No significant response was observed in the log number of IPVL sperm holes with age at day five and eight PAI in the young males (phase 1), however at day two PAI ($P<0.052$) there was a trend for decline (Figure 3.9). No significant response was observed in the young males (phase 2) in eggs collected two days PAI, however a significant ($P<0.05$) negative response was observed in eggs collected five (Figure 3.10) and eight (Figure 3.11) days PAI. In the young males the log number of IPVL sperm holes in oviposited eggs decreased as they aged, which might link to the age-related decline in egg fertility in broiler breeder flocks. Bramwell *et al*. (1996b) and Stains *et al*. (1998) both observed a decrease in the numbers of IPVL sperm holes with ageing flocks. Hazary *et al*. (2000) studied four broiler breeder flocks from 30 to 50 WOA, and observed the median number of IPVL sperm holes in samples of eggs fall from around 200 at peak production to less than 20 at 55 WOA, whilst the mean proportion of fertile eggs laid by the flocks fell from 94% at peak production to around 79% at 55 WOA.

Although there was a general decrease in the number of IPVL sperm holes with age in the young males, eggs collected two days PAI did not have a significant lower number of IPVL sperm holes from when the males were 27 WOA to when they were 60 WOA. After copulation or insemination the sperm storage tubules of the hen become full of fecund spermatozoa (Bakst *et al*., 1994) which are released sequentially providing fertile eggs for a limited period of time (Brillard, 1993). Therefore as long as the mating frequency is high enough, the sperm storage tubules will always be full of fecund spermatozoa and the egg fertility may not decline throughout the production period. Therefore the age-related decline in fertility may be due to lesser frequency of mating. Spiking and intra-spiking are two management techniques used by many breeder operations worldwide to increase the fertility of the flock during the second half of the breeding season (Casanovas & Wilson, 1999; Casanovas, 2002). By replacing all of some older males with young ones, flock fertility is seen to increase (Casanovas, 2002), this increase in fertility could be due to an increase in completion between the males resulting in an increase in mating frequency. Another management technique could be to increase the mating ratio within the flock.

Hazary *et al*. (2001) showed that the mean number of IPVL sperm holes increased linearly as the mating ratio of males to females increased.

The old males showed no response in the log number of IPVL sperm holes with age. The lack of response in the old males could be because they were only tested over an 11 week period.

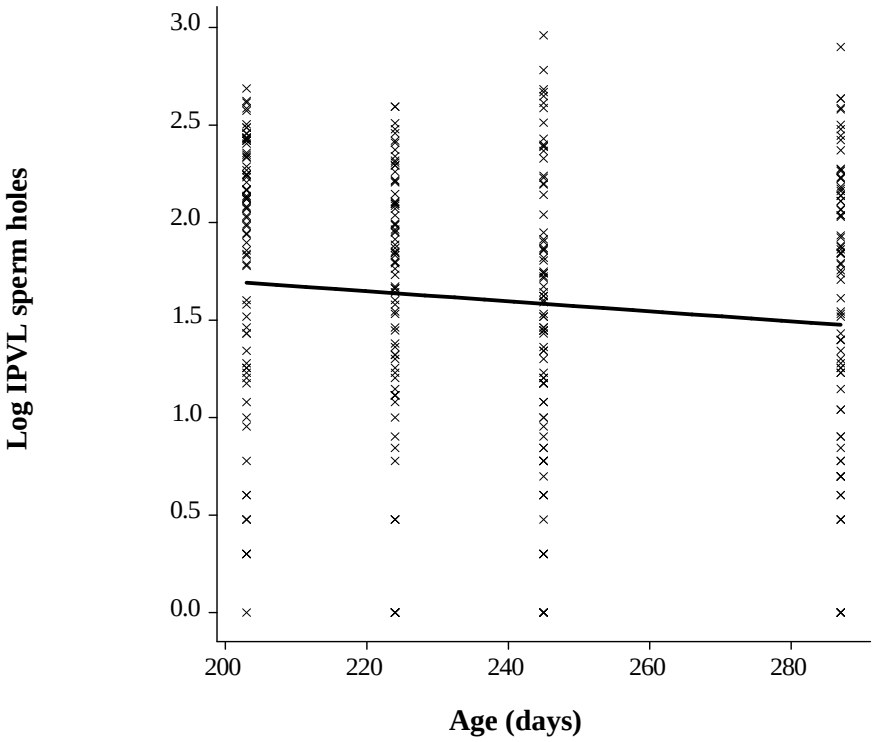

Figure 3.9 *Log number of IPVL sperm holes in eggs collected two days PAI with age in young males (phase 1)*

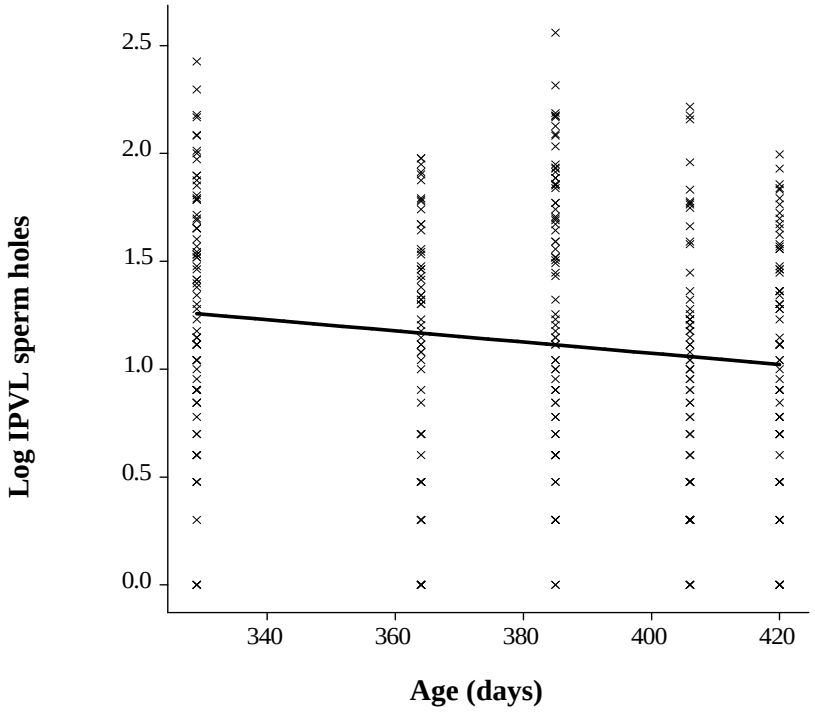

Figure 3.10 *Log number of IPVL sperm holes in eggs collected five days PAI with age in young males (phase 2)*

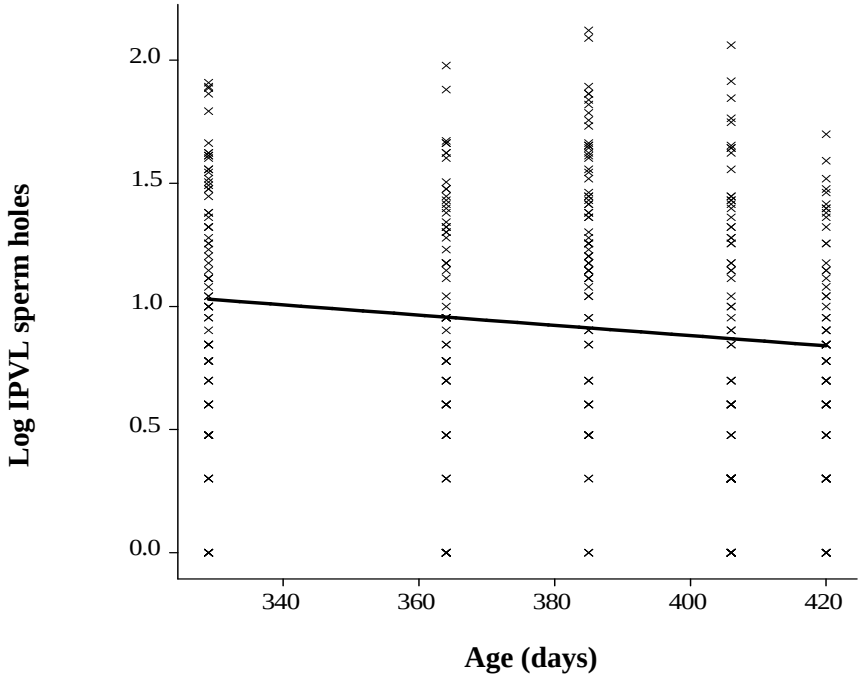

Figure 3.11 *Log number of IPVL sperm holes in eggs collected eight days PAI with age in young males (phase 2)*

A significant (P<0.001) negative response was observed in the mean number of IPVL sperm holes with days PAI in both the young and old males (Figure 3.12). After insemination the hen's sperm storage tubules fill with fecund spermatozoa, which are released sequentially providing fertile eggs for a limited period. As the days PAI increase the average number of IPVL sperm holes decrease, this relationship was similar to that observed by Wishart (1987) as well as Tyler & Bekker (2012) in spermatozoa trapped in the OPVL of oviposited eggs. After eight days PAI the average number of IPVL sperm holes in both the young and old males was still higher than six, meaning that eggs were still fertile after eight days PAI.

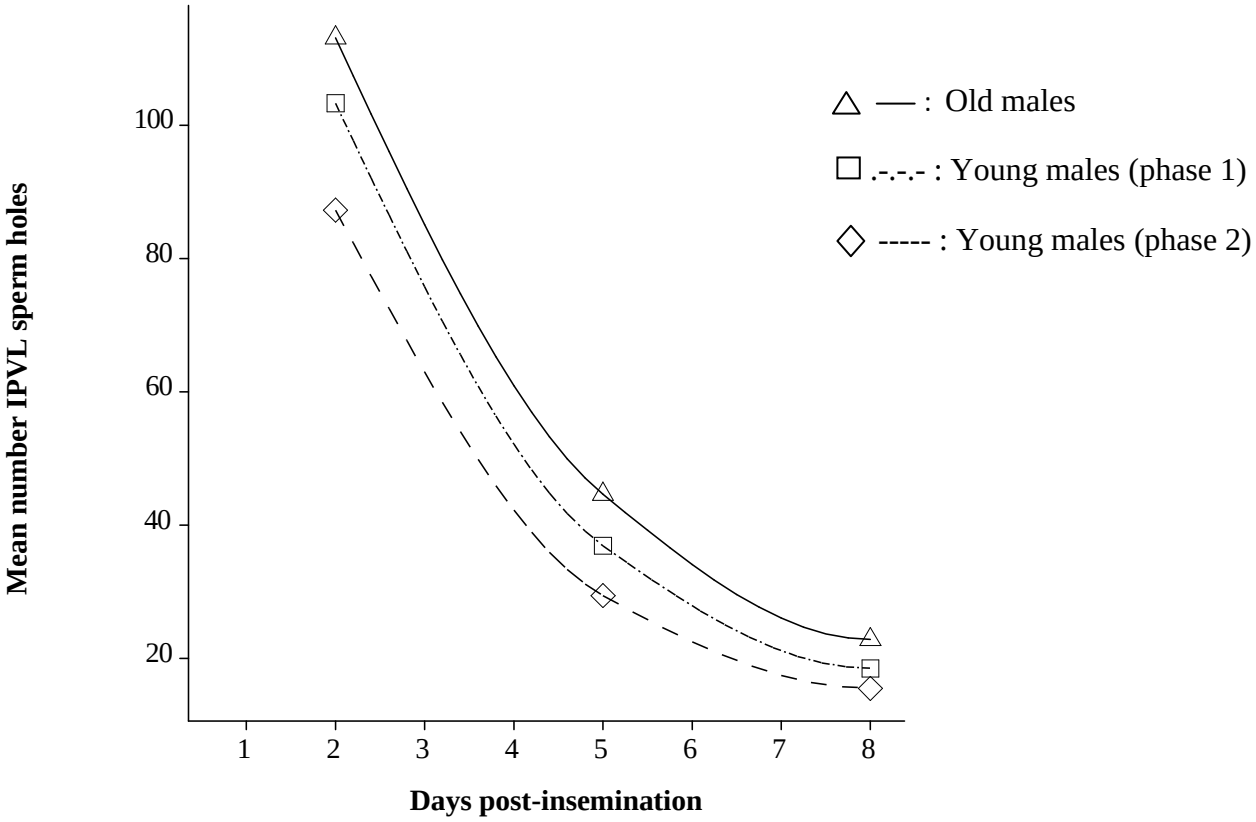

Figure 3.12 *Log number of IPVL sperm holes of eggs obtained from laying hens on days two, five, and eight PAI*

3.3.4 Cumulative Protein Intake

No significant difference in sperm concentration or sperm mobility was observed between young (phase 2) and old males at 60 WOA on the low, medium and high cumulative protein intakes. This means that sperm concentration and sperm mobility were not affected when males were subject to the different CP intakes throughout the breeding period as compared to the last 8 weeks of the breeding period. There was however a significant difference in the log number of IPVL sperm holes between the young (phase 2) and old males on the medium ($P<0.05$) and high ($P<0.001$) cumulative protein intakes, with the old males showing a significantly higher log number of IPVL sperm holes than the young males (phase 2) at the same age.

There was no significant difference in the log number of IPVL sperm with cumulative protein intake by the end of production. This same result for the low, medium and high cumulative protein intakes between the young and old males was also observed at each day PAI.

3.3.5 Egg fertility

The results of the Pearson Chi-Square analysis of fertile and non-fertile eggs (Table 3.4) showed that CP intake had no significant effect on the number of fertile and non-fertile eggs in the young males phase 1. In the young males phase 2, eggs with more than 2 sperm holes (50% probability of being fertile), a significant effect of CP intake was observed – with more non-fertile eggs from males fed 13.7 g CP/bird/day (lower CP intake) at 52 WOA in eggs collected 2 DPI, and at 55 WOA in eggs collected 5 DPI. In eggs with more than six sperm holes (maximum fertility) CP intake had a significant effect on the numbers of non-fertile eggs in the young males phase 2, with a greater count of non-fertile egg with lower CP intake at 55 WOA in eggs collected 5 and 8 DPI. The old males, in eggs with more than six sperm holes, there was a significant effect of CP intake on egg fertility at 57 and 60 WOA in eggs collected 8 DPI, with greater non-fertile eggs from males fed 14 g CP/bird/day.

In all of the above results the low CP level was seen to have significantly less fertile eggs than the medium and high CP levels. Thus towards the end of production (52-60 WOA) it was seen that sperm collected from males fed the low CP diet showed a significantly lower amount of fertile eggs compared to sperm collected from males on the medium and high CP diets. Males on the medium and high CP diets showed similar counts of fertile and non-fertile eggs throughout the breeding period. There is a known age related decline in fertility towards the end of production, which is consistent with the results seen here in the males on the low CP diet. Therefore, feeding lower levels of CP may result in decreased fertility towards the end of production, however these results were see mostly in eggs collected 5 and 8 DPI and not 2 DPI, therefore, as long as the mating frequency of the flock remains high to refill the sperm storage tubules frequently (less than 5 days between matings) it may be possible to feed a lower level of CP to males towards the end of the production period, without observing a decrease in egg fertility.

Table 3.4 Chi-square values and probabilities of egg fertility (> 2 and > 6 sperm holes) in young and old males.

			Egg fertility			
			> 2 sperm holes		> 6 sperm holes	
	Age (weeks)	DPI	χ^2-value	P-value	χ^2-value	P-value
Young males (phase 1)	29	2	0.12	0.94	1.01	0.61
		5	0.84	0.66	0.62	0.74
		8	0.41	0.82	2.75	0.25
	32	2	2.86	0.24	5.68	0.06
		5	1.89	0.39	2.36	0.31
		8	0.04	0.63	1.23	0.54
	35	2	0.63	0.73	1.29	0.53
		5	1.12	0.57	0.18	0.91
		8	0.13	0.94	0.25	0.88
	41	2	0.29	0.87	4.36	0.11
		5	0.54	0.77	0.48	0.79
		8	1.08	0.58	1.49	0.48
Young males (phase 2)	47	2	4.66	0.10	4.95	0.08
		5	5.20	0.07	2.67	0.26
		8	0.56	0.76	0.24	0.89
	52	2	7.32	0.03*	4.56	0.10
		5	0.61	0.74	0.75	0.69
		8	3.18	0.20	0.18	0.91
	55	2	5.84	0.54	5.04	0.08
		5	14.45	0.001*	6.21	0.05*
		8	2.12	0.35	7.18	0.03*
	58	2	0.03	0.99	1.18	0.56
		5	0.11	0.95	0.44	0.80
		8	3.74	0.15	1.91	0.38
	60	2	1.23	0.54	1.43	0.49
		5	2.15	0.34	1.52	0.47
		8	0.40	0.82	0.07	0.96
Old males	54	2	1.00	0.61	0.99	0.61
		5	5.01	0.08	2.96	0.23
		8	2.88	0.24	2.93	0.23
	57	2	2.37	0.31	5.85	0.05*
		5	0.12	0.94	0.89	0.64
		8	0.72	0.70	6.92	0.03*
	60	2	0.30	0.86	0.14	0.93
		5	0.94	0.63	0.49	0.78
		8	0.32	0.85	6.36	0.04*

* $P<0.05$

3.3.6. Testes Weight

There was no significant difference in the average testes weight between CP treatments. Wilson *et al*. (1987a, b) also observed no significant difference between average testes weight in males fed diets containing 9 to 18% CP. From the paired T-tests the null hypothesis was rejected for birds on the low and high ($P<0.05$) CP treatments, meaning the average weights for the left and right testis were not equal, however for birds on the medium CP treatment the null hypothesis was accepted ($P=0.083$), meaning the average weights for the left and right testis were equal. Therefore males being fed 17 g CP/bird/day from 52 to 63 WOA showed more symmetrical left and right testes weights, while males being fed either 14 or 18.9 g CP/bird/day for the same period of time had different left and right testes weights. Wolanski *et al*. (2004) described the left testis as larger or heavier than the right testis in 48 and 63-week-old broiler breeders. This was also the case in this study, with males having a larger left testis compared to the right testis. Tyler & Gous (2009) found directional asymmetry in broiler breeders, where the left testis was significantly heavier than the right in birds photostimulated at 56, 77, 98,119 and 147 days old. Without having been able to measure testes weight of the males earlier in the breeding cycle it is impossible to determine if CP level had any effect on the symmetry of the testes. During the expected end of production regression, the testes may have regressed at different rates causing asymmetry of the testes weights.

3.5 CONCLUSION

The three levels of CP intake fed to broiler breeder males from 27 to 63 WOA did not affect the BW's of the birds between each group, and the average bird weight remained close to that of the primary breeder. It can be concluded that the low CP treatment (12.6 – 14.0 g CP/bird/day) was still a sufficient amount of CP for maintenance and growth in the males. Therefore producers could feed male breeders a ration lower in CP without having to worry about decreased male growth and muscle deposition. The level of CP intake was

also seen to have no significant effect on the sperm concentration as well as the sperm mobility of the males. A number of authors have reported no effect of dietary CP level on sperm concentration, and due to the strong genetic contribution towards sperm mobility it was unlikely that CP level would have an influence. However, to the best of my knowledge the effect of CP level on sperm mobility has never been assessed before. Birds receiving the medium CP intake did however show the highest sperm mobility values. Therefor feeding males between 15.2 and 17.0 g CP/bird/day may result in a slight increase in male fertility.

Although CP intake did not significantly affect the sperm concentration and sperm mobility of the males, it did have a significant positive effect on the number of IPVL sperm holes seen in oviposited eggs. The number of sperm holes was seen to increase with the level of CP intake during the latter part of the production phase (42-60 WOA) and no difference was seen during the early production phase (27-41 WOA). However, the number of IPVL sperm holes was still high enough for the eggs to be fertile across all CP intakes and therefor feeding a low CP diet (12.6 – 14 g CP/bird/day) does not cause the males to become significantly less fertile.

The aim of this thesis was to assess the effects of dietary CP level on fertility in young and old male broiler breeders, in order to make recommendations to maximize male breeder fertility whilst also lowering feeding costs to producers. Males are often fed a female ration containing a higher amount of CP than what is required by the male. Sex-separate feeding systems make it possible to feed males and females separately, thus allowing males to be fed a diet lower in CP thus eliminating the negative effects associated with feeding high levels of CP as well as decreasing the cost of the feed due to the high cost of CP. From this study it was seen that dietary CP level had more of an effect on male fertility during the latter part of the production phase compared to earlier in the production phase. CP level did not affect sperm concentration nor sperm mobility, and although it did have an effect on the number of IPVL sperm holes and therefore fertility, males receiving between 12.6 and 18.9 g CP/bird/day remained fertile throughout the production period. Due to the decrease in IPVL sperm holes with days post-insemination or mating it can be suggested that as

long as the mating frequency of the flock is high throughout the breeding period, the hen's sperm storage tubules will be full of fecund spermatozoa thus providing fertile eggs throughout the production cycle. Flock management such as correct mating ratio as well as techniques such as inter and intra-spiking can be employed in order to achieve a high enough mating frequency throughout the production cycle. Therefore, producers may feed males a separate ration lower in CP (12.6 – 14 g CP/bird/day) without having a decrease in male fertility as long as the mating frequency of the flock remains high. There was however, a tendency for a superior response in fertility from birds on the medium CP intake (15.2, 16.6 and 17.0 g CP/bird/day for the young males phase 1, phase 2 and old males respectively). Therefore the recommendation emanating from this research is to provide male broiler breeders with an intake of 15.2 g CP/bird/day from 27-41 WOA, and between 16.6 and 17 g CP/bird/day for the remainder of the breeding period (42-62 WOA).

CHAPTER 4

GENERAL CONCLUSIONS

With continual genetic improvements in the broiler industry, and broiler growth traits being negatively correlated to fertility traits, it is essential for the broiler breeder industry to improve breeder management strategies in order to maintain or improve fertility and prevent the potential decline in reproductive performance of males and females. The aim of this thesis was to review some older as well as more recent techniques in predicting male broiler breeder fertility and use them to determine the optimum level of dietary CP required to achieve maximum fertility in males during the production phase.

High BWs are seen as a major cause in the reduction of male fertility; therefore commercial breeder producers employ certain feeding strategies in order to control male BWs. However, there is variation among producers as to which feeding strategies they employ, specifically to the level of CP in the diet, degree of feed restriction and age at commencement of restriction. Management during rearing is an important factor affecting fertility during production, especially in terms of BW and conformation of the birds at maturity. It has been seen that differences in CP levels during rearing did not affect male fertility measures during production, and that the level of ME intake during rearing had more of an effect on body composition and growth rate than CP intake did. There has been much research conducted on the effect of different levels of CP on male breeder fertility, however the findings have been inconsistent. Some authors report no significant effect of CP on male fertility measures (sperm concentration, semen volume, number of spermatozoa per ejaculate, sperm viability and testes weight) however; others report high and low levels of CP adversely affecting these measures. Fertility measures in these studies were some older methods of predicting male fertility, in this study some more modern methods were used to predict male fertility.

Measuring the number of IPVL sperm penetration holes in oviposited eggs, as well as measuring the mobility of sperm collected from males are two more modern predictors of fertility. Both methods have been shown to have a significant effect on egg fertility and have been shown to be reliable indicators of male fertility. They can also be used to measure and improve the breeding efficiency of broiler breeder flocks. This study showed that as the level of CP in the diet increased so did the number of IPVL sperm holes, however it has been shown that maximum egg fertility occurs if six or more sperm penetration holes are seen around the germinal disk of the egg, and therefore it was seen that males receiving the low CP diet did not have significantly less fertile eggs compared to males receiving the high CP diet. No significant effect of CP level on sperm mobility was seen in this study. This could be due to the strong genetic contribution to sperm mobility. Sperm mobility has been shown to be a heritable trait; however, there is indication that it may only be transmitted maternally; therefore selective breeding of high mobility phenotype males may not increase the chances of their progeny having a high sperm mobility phenotype. This study predicted the fertility of individually caged male broiler breeders as it would be difficult to keep track of, as well as collect and analyse semen from males in a breeding flock. However results may be different if the same study was performed on males in breeding flock, since males will have more work to do in terms of natural mating as well as competition with other males.

Breeder flock fertility is seen to decline during the latter weeks of production, this is mainly attributed to excessive BW and poor physical condition of older males. It has been seen that an increase in feed allocation (increased ME) during this stage could restore late fertility. It was also reported that for males the quantity of feed during production played a more important role in late fertility than the feed allocation program during rearing. This study showed that as long as the mating frequency of the flock remains high throughout the breeding period, the number of sperm penetrating the IPVL will remain high, thus providing fertile eggs throughout the production cycle and potentially correcting the decline in fertility during the latter weeks of production. Spiking is one management

strategy which may be employed to increase the mating frequency of a flock towards the end of production.

Often males are fed the same feed as the females, however their nutritional requirements differ. Males require less CP in their diet compared to females which have a CP requirement for egg production that is higher than that of spermatogenesis. This study confirms that male broiler breeders may be fed a diet lower in CP than that of the diet fed to females. Overfeeding CP has been shown to have a negative impact to the birds as well as the environment. Crude protein is also one of the most costly components in poultry feed, and feed is the most costly component of commercial poultry production (D Van Den Berg, 2014, pers comm.), therefor if male breeders can be fed a lower CP diet, producers can save on feed costs without compromising male fertility. An average sized breeder farm houses around 45 000 females and 4500 males (D Van Den Berg, 2014, pers. comm.), feeding each male an average of 142 g/bird/day for a production cycle of 40 weeks equals a total feed consumption of 180 tons. A female breeder ration costs around R3614 per ton, and a male ration (lower in CP) R3148 per ton (K Jordaan, 2014, pers. comm.). Therefore feeding males a male feed ration rather than a female ration results in a saving of around R466 per ton and a total saving of R83 880 per cycle. There will be additional costs for producers feeding males a separate ration, such as an extra bulk feed tank for the male feed, however one bulk tank is sufficient per breeding farm. Another alternative for smaller breeding operations is to buy individual feed bags.

The results of this study have shown that feeding male broiler breeders a lower level of CP did not negatively affect their fertility, therefore males can be fed a diet lower in CP. However one of the challenges facing feed manufacturers is being able to formulate a bulk male ration below 120 g CP/kg and at the moment producers who feed males a separate feed are using a ration containing around 125 g CP/kg (D Van Den Berg, 2014, pers. comm.).

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